The opinion in support of the decision being entered today is not binding precedent of the Board.

Paper [redacted]

By: Trial Section Merits Panel
Board of Patent Appeals and Interferences
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

MAILED
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PAT. & T.M. OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

WEN-HWA LEE
and EVA Y-H.P. LEE

Junior Party,
(U.S. Patent 5,998,134)

v.

THADDEUS P. DRYJA,
STEPHEN FRIEND and DAVID W. YANDELL

Senior Party,
(Application 09/387,158)

Patent Interference No. 105,182

Before: TORCZON, SPIEGEL and LANE, Administrative Patent Judges.

SPIEGEL, Administrative Patent Judge.[1]

DECISION - MOTIONS - Bd.R. 125(a)

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[1] As part of the Board's efforts under the Government Paperwork Elimination Act, signatures on papers originating from the Board are being phased out in favor of a completely electronic record. Consequently, in this case papers originating at the Board will not have signatures. The signature requirements for the parties have not changed. See e.g., 37 C.F.R. § 10.18.

I. Introduction

Interference 105,182 was declared on 22 September 2004 between junior party WEN-HWA LEE and EVA Y-H.P. LEE ("**Lee**") and senior party THADDEUS P. DRYJA, STEPHEN FRIEND and DAVID W. YANDELL ("**Dryja**"). Lee is involved in the interference on the basis of U.S. Patent 5,998,134 ("the '134 patent"), issued 7 December 1999, based on U.S. application 08/482,627 ("the '627 application"). Dryja is involved in the interference on the basis of U.S. application 09/387,158 ("the '158 application") filed 31 August 1999. The subject matter of the interference is defined by one count, i.e., Lee '134 patent claim 1 or Dryja '627 application claim 22, and is directed to a method of detecting a mutated retinoblastoma ("RB") nucleic acid in a sample by using an isolated cDNA which encodes a full length, wild-type RB protein as a hybridization probe. Lee '134 patent claims 1-4 and Dryja '158 application claims 22, 23 and 49 were designated as corresponding to the count. [Paper 1.]

Among the motions filed during the motion phase of the interference was Dryja revised motion 2. Dryja revised motion 2 sought judgment that Lee '134 patent claims 1-4 are barred on the basis of interference estoppel or res judicata because Lee received an adverse decision in prior interferences 103,426 ("the '426 interference") and 104,259 ("the '259 interference") (Paper 33). Dryja revised motion 2 was **granted** for reasons set forth in the "DECISION - PRELIMINARY MOTIONS - Bd.R. 125(a)" (Paper 76) issued concurrently with this judgment and is a dispositive motion. As a result of granting Dryja revised motion 2, Lee no longer has any patentable claims corresponding to the sole count in the interference. Since Lee no longer has any

patentable claims corresponding to the sole count in the interference, it is appropriate to enter judgment at this time.

II. Order

Therefore, based on the foregoing, it is

ORDERED that judgment on priority as to Count 1 (Paper 1, p. 5) is awarded against junior party WEN-HWA LEE and EVA Y-H.P. LEE;

FURTHER ORDERED that junior party WEN-HWA LEE and EVA Y-H.P. LEE is not entitled to a patent containing claims 1-4 (corresponding to Count 1);

FURTHER ORDERED that if there is a settlement agreement and it has not already been filed, attention is directed to 35 U.S.C. § 135(c) and 37 CFR § 1.661; and,

FURTHER ORDERED that a copy of this judgment (Paper 77) and of the decision on motions (Paper 76) be given appropriate paper numbers and entered into the file records of U.S. Patent No. 5,998,134 and U.S. application 09/387,158.

\ Richard Torczon \
RICHARD TORCZON
Administrative Patent Judge

\ Carol A. Spiegel \
CAROL A. SPIEGEL
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

\ Sally Gardner Lane \
SALLY GARDNER LANE
Administrative Patent Judge

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I. Introduction

This is a decision on the remaining motions in interference 105,182. Lee motion 1 attacks the benefit for the purpose of priority accorded to Dryja. Lee motion 2 seeks judgment that Dryja's claims are unpatentable under 35 U.S.C. §§ 102(b)/103(a). Dryja revised motion 2 seeks judgment that Lee's claims are barred on the basis of estoppel or res judicata. Dryja motion 4 seeks judgment that Lee's claims are unpatentable under 35 U.S.C. § 102(a) and/or 103(a). Dryja revised motion 2 is **granted**. Since Dryja revised motion 2 is dispositive, the remaining motions are dismissed as moot.

II. Background[2]

A. Genetic predisposition to disease

A *genome* is all the hereditary material possessed by a living organism. The basic unit of hereditary material is the gene. A *gene* is an ordered sequence of nucleotide bases, i.e., DNA (or, for some viruses, RNA), that encodes a functional product, e.g., a protein. Typically, a gene has a coding region which determines the sequence of amino acids in the protein product. The gene includes regions preceding and following the coding region, as well as intervening sequences (*introns*) between individual coding segments (*exons*).

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[2] This "Background" section is intended as merely a brief overview to provide a context for discussion of the subject matter of the interference and is not intended as a comprehensive treatment of either genetic predisposition to disease generally or to retinoblastoma specifically. See generally,

U.S. Patent 5,998,134 (Ex 1142, cc. 1-5);

MOLECULAR BIOLOGY AND BIOTECHNOLOGY: A COMPREHENSIVE DESK REFERENCE, R. Meyers, ed., VHC Publishers, Inc., New York, NY (1995), pp. 428-431 and 817-820 (copy enclosed);

MOLECULAR CELL BIOLOGY, second edition, Darnell et al., eds., Scientific American Books, Inc., New York, NY (1990), p. 996 (copy enclosed); and

MICROBIOLOGY: AN INTRODUCTION, Tortora et al., The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA (1982), pp. 201-214 (copy enclosed).

In a process called *transcription*, much of the genetic information in DNA is copied or encoded by a complementary base sequence of RNA. The RNA message is then used by the cell to synthesize specific proteins through a process called *translation*. A *promoter* (a DNA sequence which controls the expression of a gene) directs the synthesis of the RNA copy for the gene and splicing signals control the precise removal of the introns. Each end of the spliced RNA (now called *mRNA*) is further modified before it is used as a template for protein synthesis.

The human genome includes up to 10^5 genes. Except for identical twins, no two people have exactly the same DNA sequence in their genes. Since less than 5% of the genome contains protein coding sequences, most DNA variations between people have no apparent effect. Other DNA differences affect a person's *phenotype* (observable characteristics) and result in normal variable characteristics typically found in natural populations, such as different eye and hair colors. A *wild-type* gene or DNA is a gene or DNA that confers a phenotype considered to be a "normal" type commonly found in natural populations. For example, "normal" or "wild-type" genes for hair and eye pigment color might confer red hair and blue eye coloring or brown hair and brown eye coloring. Thus, there can be multiple "wild-types" of a gene. However, a small percentage of DNA differences result a phenotype that is considered abnormal. For example, the genes that confer hair, skin and eye pigment color in albinos are <u>not</u> wild-type genes, since albinos have milky or translucent skin, white or colorless hair, and eyes with pink irises and deep-red pupils which is a phenotype not commonly found in nature. In other words, a wild-type gene is a non-mutated gene that is commonly found

in nature, whereas a mutated or abnormal gene is one that is not commonly found in nature.

Several thousand of the genes in the human genome can cause disease if altered (*mutated*) in some way. A gene mutation that increases an individual's susceptibility to a certain disease or disorder is called a *predisposing mutation*. A gene alteration which occurs in the reproductive cells of sexually reproducing organisms (i.e., in egg or sperm cells) becomes incorporated in the DNA of every cell in the organism's body and can be passed to subsequent generations is called a *germline mutation*. *Somatic mutations* are DNA mutations that occur after conception. Somatic mutations can occur in any of the cells of the body except the germ cells (egg or sperm) and therefore are neither inherited nor passed on to subsequent generations.

The mutation may be a deletion, insertion, duplication, inversion or some other rearrangement of DNA and may involve a single nucleotide or an entire chromosome.[3] For example, if the mutation occurs in the protein coding region of a gene, the amino acid sequence of the final gene product may be changed or even shortened if a premature *stop codon* (a triplet nucleotide sequence in mRNA that signals termination of protein synthesis) is created. A mutation can also affect the promoter or processing signals, e.g., splicing signals. As a result, the protein product of the mutated gene may be an abnormal (e.g., nonfunctional or truncated) protein or the protein may not be made at all or the wrong amount of the protein may be made or the protein may be

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[3] A chromosome is a rod-shaped grouping of coiled strands of DNA and proteins in the cell nucleus that contain specific genes carrying hereditary information. Most multicellular organisms have several chromosomes, which together comprise the genome. Sexually reproducing organisms, e.g., humans, have two or more copies of each chromosome, one from each parent.

made at the wrong time in the organism's development.

B. Retinoblastoma

Retinoblastoma ("RB"), the most commonly occurring malignant tumor of the developing retina in infants and children, is a classic example of gene mutations that predispose an individual to a specific disease or tumor. Genetic evidence suggests that RB occurs as the result of two mutational events. Genetic evidence further suggests that the human retinoblastoma gene is located within chromosome 13 and that absence or inactivation of the retinoblastoma gene is the primary cause of RB. Specifically, deletions in the retinoblastoma gene have been associated with retinoblastoma tumor.

In hereditary RB the first mutation in the RB gene is a germline mutation, i.e., the mutation occurs in the reproductive cells (eggs or sperm) of a parent, and thus is present in all cells of a child. There is a 50% risk that a child of a patient with RB will receive an abnormal gene for RB and a 90% chance that a child with the mutated gene will develop an RB tumor. There is also an increased risk for development of secondary tumors, particularly osteosarcoma. A second single mutation in the somatic target cell (retina cell for RB or bone cell for osteosarcoma) is required for development of the tumor. These tumors might be expected to occur at an early age and to be bilateral (i.e., present in both eyes). In non-hereditary RB, both mutations occur in the same somatic cell (retina cell or bone cell) after conception. Thus, these tumors might be expected to occur at a relatively later age and to be unilateral.

Therefore, in order to determine the genetic predisposition in a fetus or the susceptibility at a later age of developing RB, it is important to identify the exact location

of the RB gene and to isolate, clone and sequence the full-length, wild-type RB gene. Isolation, cloning and sequencing of a full-length, wild-type RB gene provides the nucleic acid sequence and predicted amino acid sequence of a normal functional RB protein product. It also provides a standard against which to determine the presence of mutations, e.g., deletions, in a sample nucleic acid sequence which alter the nucleic acid sequence of the sample's RB protein product such that either no, or an "abnormal" (e.g., non-functional or truncated), RB protein product is produced by the mutated gene.

The subject matter of this interference generally relates to isolation and identification of RB genes in order to diagnose RB or a predisposition thereto. In particular, the subject matter of the interference is directed a method of using a cDNA clone of a full-length, wild-type RB gene as a hybridization probe to detect the presence of a mutated RB gene nucleic acid in a test sample. Hybridization is a technique in which the degree of sequence identity between nucleic acids can be determined. The technique measures the ability of one nucleic acid strand (e.g., a cDNA clone of a full-length, wild-type RB gene or "probe") to hybridize (bind through complementary nucleic acid base pairing) with another nucleic acid strand (e.g., DNA or RNA from a cell sample of a child being tested for the presence of a mutated RB gene). For example, a labeled probe specific for the normal RB gene (a cDNA clone of a full-length, wild-type RB gene labeled with a detectable marker) may be hybridized to a control DNA sequence from a "normal" individual, i.e., an individual without RB disease, and to a sample nucleic acid, e.g., DNA, from an individual being tested for the presence of a mutated RB gene. The absence of hybrid formation between the probe and the sample

DNA (no marker detected) or the presence of hybrids of a smaller size between the probe and the sample DNA compared to hybrids obtained between the probe and the control DNA (less marker detected in the test DNA/probe hybrid vis-a-vis the amount of marker detected in the control DNA/probe hybrid) is an indication of a mutated RB DNA (gene) in the test individual.

III. Findings of fact (FF)

The following findings of fact are supported by a preponderance of the evidence.

1. The junior party is WEN-HWA LEE and EVA Y-H.P. LEE ("**Lee**").

2. Lee is involved in the interference on the basis of U.S. Patent 5,998,134 (the "'134 patent," Ex 1142), issued 7 December 1999, based on U.S. application 08/482,627 ("the '627 application"), filed 7 June 1995.

3. The '134 patent has been accorded benefit for the purpose of priority of
   (i) U.S. application 07/951,947 filed 28 September 1992, and
   (ii) U.S. application 07/108,749, filed 15 October 1987.

4. Lee's real parties-in-interest are
   (a) its assignee, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, and
   (b) its exclusive licensee, CANJI, INC., a wholly owned subsidiary of Schering-Plough Corporation.

5. The senior party is THADDEUS P. DRYJA, STEPHEN FRIEND and DAVID W. YANDELL ("**Dryja**").

6. Dryja is involved in the interference on the basis of U.S. application 09/387,158,

(the "'158 application") filed 31 August 1999.

7. The '158 application has been accorded benefit for the purpose of priority of

(i) U.S. application 08/255,572, filed 8 June 1994,

(ii) U.S. application 07/951,342, filed 25 September 1992,

(iii) U.S. application 07/728,756, filed 8 July 1991,

(iv) U.S. application 07/300,667, filed 23 January 1989,

(v) U.S. application 07/146,525, filed 21 January 1988, and

(vi) U.S. application 06/895,163, filed 11 August 1986.

8. Senior party's real parties-in-interest are

(a) MASSACHUSETTS EYE AND EAR INFIRMARY,

(b) THE WHITEHEAD INSTITUTE, and

(c) BAYER HEALTHCARE LLC.

9. The subject matter of the interference is defined by one count, which reads:

Count 1

Claim 1 of Lee (5,998,134) or claim 22 of Dryja (09/387,158).

10. Claim 1 of the Lee '134 patent reads (emphasis added):

A method of detecting a mutated retinoblastoma ("RB") nucleic acid in mammals, the method comprising the steps of:
(i) hybridizing **an isolated, full-length, wild-type RB cDNA** probe to a cell sample, **wherein the full-length, wild-type RB cDNA** probe **has the nucleotide sequence depicted in Figure 7**; and
(ii) detecting a mutated RB nucleic acid.

11. Claim 22 of the Dryja '158 application reads (emphasis added):

A method of detecting a mutated retinoblastoma ("RB") nucleic acid, the method comprising the steps of:
(i) hybridizing **an isolated full-length, wild-type RB cDNA** probe

to a mammalian cell sample; and
(ii) detecting a mutated RB nucleic acid.

12. The claims of the parties are:

Lee	1-4
Dryja	22-31, 49 and 50-59

13. The claims of the parties which correspond to Count 1 are:

Lee	1-4
Dryja	22, 23 and 49

14. The claims of the parties which do not correspond to Count 1, and therefore are not involved in the interference, are:

Lee	none
Dryja	24-31 and 50-59

["DECLARATION," Paper 1.]

Other findings of fact follow below.

IV. Dryja Revised Substantive Motion 2

Pursuant to 37 CFR §§ 41.121(a)(1) and 41.127(a)(1), Dryja seeks judgment that Lee's involved claims are barred by 37 CFR § 41.127(a)(1) (estoppel, formerly 37 CFR § 1.658(c) (2004)), based on an adverse decision against Lee in interferences 103,426 ("the '426 interference") and 104,259 ("the '259 interference") (Paper 33, p. 15). Lee opposes (Paper 39); Dryja replies (Paper 45).

Dryja's arguments that Lee is not entitled to the claims of U.S. Patent 5,858,771 (Ex 1139) based on estoppel are moot because the '771 patent is not involved in this interference. To the extent Dryja is seeking relief with respect to the uninvolved '771 patent claims, the motion is **dismissed**.

A. The parties' positions

According to Dryja, Lee should not be allowed to obtain patent rights in subject matter that Lee lost in the '426 or '259 interference or on subject matter that could have been put in issue during either of those interferences (Paper 33, p. 26, penultimate ¶). Dryja argues that Lee had notice of certain subject matter commonly disclosed in the applications involved in the '426 and '259 interferences, including methods of using RB nucleic acids as hybridization probes (Paper 33, p. 4). Dryja further argues that Lee could have put in issue priority of any commonly disclosed subject matter in the '426 and/or '259 interference but did not. Since Lee requested and received adverse judgments in both the '426 and '259 interferences, Dryja contends that Lee is estopped from raising the issue of commonly disclosed subject matter now.

Dryja specifically argues that, having lost its claim to an isolated cDNA encoding a full-length, wild-type RB protein in the '426 interference, Lee is estopped from claiming methods of using the isolated cDNA as a hybridization probe to detect mutated RB nucleic acids because this is the exact utility described in Dryja's applications for the isolated cDNA (Paper 33, pp. 18-19). Dryja further argues that Lee should have raised the issue of priority of using an isolated full-length, wild-type RB cDNA as a hybridization probe to detect mutated RB nucleic acids in the '259 interference (Paper 33, pp. 25-26).

In its opposition, Lee contends that Dryja's motion is procedurally defective because it does not include a comparative claim chart. Lee further contends that Dryja's motion is substantively defective not only because Dryja has not shown that Lee

could have earlier raised the issue of priority of using an isolated full-length, wild-type RB cDNA as a hybridization probe to detect mutated RB nucleic acids, but also because Dryja had notice that Lee considered an isolated full-length, wild-type RB cDNA to be patentably distinct from the subject matter of the '426 interference (Paper 39, p. 13; p. 18, ¶¶ 2-3; p. 19, ¶ 3; p. 20, ¶ 1; p. 21, ¶ 3).

B. A brief historical background of interference estoppel

Historically, four types of estoppel have been raised in interference proceedings: (1) estoppel by dissolution, (2) estoppel by judgment, (3) equitable estoppel, and (4) estoppel for failure to move. Woods v. Tsuchiya, 754 F.2d 1571, 1578, 225 USPQ 11, 15-16 (Fed. Cir.), cert. denied, 474 U.S. 825 (1985).

> *Estoppel by dissolution* prevents a junior party who had access to the senior party's application from obtaining claims to common patentable subject matter after an interference is dissolved. *Estoppel by judgment* prevents a losing party in a previous interference between the same parties from making any claim (1) not patentably distinct from the counts in issue in that interference, or (2) which reads on the disclosure of the winning party to which the losing party had access. *Equitable estoppel* prevents the winning party in a previous interference terminated by judgment (or the senior party in an interference which ends in dissolution) from claiming patentably distinct subject matter to which the other party did not have access. *Estoppel for failure to file a motion to amend* would prevent a party who fails to file a timely interlocutory motion to amend from later claiming subject matter which could have been added by such a motion. [Id., citations omitted.]

This case involves an estoppel based on a failure to move. See In re Kroekel, 803 F.2d 705, 709, 231 USPQ 640, 643 (Fed. Cir. 1986); Ex parte Kimura, 55 USPQ2d 1537 (Bd. Pat. App. & Int. 2000).

The interference rules were revised in 1984 (37 CFR §§ 1.601 through 1.688, "the 1984 rules"), and applied to all interferences declared on or after 11 February

1985, except in a limited number of special circumstances. Under § 1.601, the rules were to be construed to secure the just, speedy and inexpensive determination of interferences. Section 1.658(c) defined estoppel, i.e.,

> A judgment in an interference settles all issues which (1) were raised and decided in the interference, (2) could have been properly raised and decided in the interference by a motion under § 1.633(a) through (d) and (f) through (j) or § 1.634, and (3) could have been properly raised and decided in an additional interference with a motion under § 1.633(e). A losing party who could have properly moved, but failed to move, under § 1.633 or § 1.634, shall be estopped to take *ex parte* or *inter partes* action in the Patent and Trademark Office after the interference which is inconsistent with that party's failure to properly move, except that a losing party shall not be estopped with respect to any claims which correspond, or properly could have corresponded, to a count as to which that party was awarded a favorable judgment.

Thus, under the 1984 rules, estoppel could be based on a failure to move. The estoppel rule was designed to encourage parties in interference cases to settle as many issues as possible in one proceeding, i.e., to secure the just, speedy and inexpensive determination of interferences.

Rule 633 permitted a party to file a variety of motions, including a

(a) motion for judgment that an opponent's claim designated as corresponding to a count was unpatentable;

(b) motion for judgment that there was no interference-in-fact; and

(c) motion to redefine the interfering subject matter by (1) adding or substituting a count, (2) amending an application claim corresponding to a count or adding a claim in the moving party's application to be designated as corresponding to a count, (3) designating an application or patent claim as corresponding to a count, (4) designating an application or patent claim as not corresponding to a count, or (5) requiring an

opponent who is an applicant to add a claim and to designate the claim as corresponding to a count.

An estoppel would not apply with respect to any claims which correspond, or which properly could have corresponded, to a count as to which a party was awarded a favorable judgment. The Notice of Final Rule, Patent Interference Proceedings, 49 Fed. Reg. 48416, 48426-27 (Dec. 12, 1984), reprinted in 1050 O.G. 385, 395 (Jan. 29, 1985) provided a number of examples of how estoppel under Rule 658(c) would apply, including

> *Example 24.* Junior party applicant AL and senior party AK both disclose separate patentable inventions "A" and "B" and claim only invention "A" in their respective applications. An interference is declared with a single count to invention A. Neither party files a preliminary motion (see § 1.633(c)(1)) to add a count to invention B. Judgment as to all of AL's claims corresponding to the sole count is awarded to junior party AL. Senior party applicant AK would be estopped to thereafter obtain a patent containing claims to invention B, because applicant AK failed to move to add a count to invention B in the interference. Junior party applicant AL would not be estopped to obtain a patent containing claims to invention B.
>
> *Example 25.* In this example, the facts are the same as in Example 24 except that the judgment is awarded as to all AK's claims corresponding to the count to senior party applicant AK. Junior party applicant AL would be estopped to obtain a patent containing claims to invention B in the interference. Senior party applicant AK would not be estopped to obtain a patent containing claims to invention B.
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> *Example 26.* Junior party applicant AM and senior party applicant AP both disclose separate patentable inventions "C", "D", and "E" and claim inventions C and D in their respective applications. An interference is declared with two counts. Count 1 is to invention C and Count 2 is to invention D. Neither party files a preliminary motion to add a proposed Count 3 to invention E. Judgment as to all AM's claims corresponding to Counts 1 and 2 is awarded to junior party applicant AM. Senior party applicant AP would be estopped to thereafter obtain a patent containing claims to invention E, because applicant AP failed to move to add a count to invention E in the interference. Junior party applicant AM would not be

estopped to obtain a patent containing claim to invention E.

Example 27. In this example, the facts are the same as in Example 26 except that judgment is awarded as to all AP's claims corresponding to Counts 1 and 2 to senior party applicant AP. Junior party applicant AM would be estopped to obtain a patent containing claims to invention E, because applicant AM failed to move to add a count to invention E in the interference. Senior party applicant AP would not be estopped to obtain a patent containing claims to invention E.

The Commissioner also pointed out that

> [p]arties in interference cases should recognize, ... that the interference estoppel provisions of § 1.658(c) have been expanded with a view to eliminating much of the *ex parte* maneuvering which has taken place in the past after an interference is eliminated. Accordingly, a party who fails to move to place a matter in issue runs a considerable risk that the party will not be able to raise the issue *ex parte* after an interference is terminated. [49 Fed. Reg. at 48441, c. 2, ¶ 2, reprinted in 1050 O.G. at 410, c. 2, ¶ 2.]

To wit,

> [t]he following comment by the CCPA in its opinion in *In re Shimer*, 69 F.2d 556, 558, 21 USPQ 161, 163 (CCPA 1934), accurately expresses the intent of the PTO in promulgating §§ 1.633(e) and 1.658(c):
>
> > It may be stated that this rule works no hardship to him who is diligent in pursuit of his rights. When an interference is declared, the files of his contestants are open to him. He has full cognizance of their disclosures and claims. So advised, it becomes his duty to put forward every claim he has. Rule 109 [i.e., Rule 1.633(e)] affords him this opportunity. If the rule be not enforced or enforceable, then delays and litigation are greatly increased. It is quite obvious that the doctrine of estoppel, as applied in these cases, results in the better conduct of the business of the Patent [and Trademark] Office and in the public good. [Id. at 48440, c. 2, ¶ 2, bracketing added.]

Rule 633(e) is not involved in this case and In re Shimer did not involve a situation where a party failed to move under § 1.633(c). However, applying the doctrine

of estoppel in cases where, as here, a party fails to avail itself of the opportunity afforded it by Rule 633(c), similarly results in more efficient conduct of the business of the USPTO and in the public good.

In 2004, the 1984 interference rules were revised as part of an overhaul consolidating and simplifying the rules governing practice before the Board of Patent Appeals and Interferences. An interference was defined as a contested case as set forth in 37 CFR §§ 41.200 through 41.208 and 41.100 through 41.158. The current interference rules apply to all interferences declared on or after 13 September 2004. Section 41.127(a)(1) recodified the former § 658(c) estoppel, i.e.,

> A judgment disposes of all issues that were, or by motion could have properly been, raised and decided. A losing party who could have properly moved for relief on an issue, but did not so move, may not take action in the Office after the judgment that is inconsistent with that party's failure to move, except that a losing party shall not be estopped with respect to any contested subject matter for which that party was awarded a favorable judgment. 37 CFR § 41.127(a)(1).

Section 41.208(a) defines the "substantive" motions (formerly "preliminary" motions under the 1984 rules) which may be filed in an interference, including motions which (1) raise a threshold issue, e.g., a no interference-in-fact issue, or (2) seek to redefine the interfering subject matter, e.g., by adding or amending a count or by changing the correspondence of claims to the count. According to § 41.207(b)(2), "A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim." "For the purposes of determining priority and derivation, all claims of a party corresponding to the count are presumed to stand or fall together. To challenge this

presumption, a party must file a timely substantive motion to have a corresponding claim designated as not corresponding to the count." 37 CFR § 41.207(b)(1).

In the Notice of Final Rule, Rules of Practice Before the Board of Patent Appeals and Interferences, 69 Fed. Reg. 49960, 49994 (Aug. 12, 2004), reprinted in 1286 O.G. 21, 55 (Sept. 7, 2004) the Director noted that § 41.207(b)

> simply formalizes the effect of the estoppel arising out of cases like *In re Deckler*, 977 F.2d 1449, 1452, 24 USPQ2d 1448, 1449 (Fed. Cir. 1992), in which a party could not subsequently seek claims that were patentably indistinct from the subject matter of the count lost in the interference.

In summary, the doctrine of estoppel serves administrative objectives of securing "the just, speedy, and inexpensive resolution of every proceeding before the Board." 37 CFR § 41.1(b). Since 1984, estoppel based on a prior judgment or on a failure to file a timely motion in a prior interference may be put in issue by filing a motion in the later interference. Estoppel benefits three separate entities: (1) the winning party, (2) the PTO and (3) the public. The winning party avoids the expense of a second interference directed to commonly disclosed subject matter, whether or not claimed by one or both parties, which the losing party could have properly put in issue and litigated in the first interference. Estoppel allows the PTO to expedite patent prosecution, maximize allocation of its resources and improve its administrative efficiency. The public gains greater certainty about who, if anyone, is entitled to a patent on commonly disclosed subject matter.

C. Estoppel based on the '426 interference

1. the '426 interference

15. On 31 January 1995, the '426 interference was declared between junior party

Lee and senior party Dryja (Ex 1004, p. 1) and 1 May 1995 was set as the time for filing preliminary statements and preliminary motions.[4]

16. Lee was involved in the '426 interference on the basis of application 07/951,947 ("the '947 application," Ex 1006), filed 28 September 1992 (Ex 1004, p. 4).

17. Dryja was involved in the '426 interference on the basis of application 07/958,290 ("the '290 application," Ex 1005[5]) filed 8 October 1992 (Ex 1004, p. 5).

18. The subject matter of the '426 interference was defined by one count, i.e.,

> Purified nucleic acid comprising a human retinoblastoma gene, or a fragment thereof comprising 15 or more bases, said nucleic acid being less than 100kb in size.
>
> [Ex 1004, p. 6.]

19. Lee '947 application claims 15, 16, 45 and 46 (Ex 1006) and Dryja '290 application claims 1-4 and 22-37 (Ex 1005) were designated as corresponding to the count (Ex 1004, p. 6).

20. Lee '947 application claim 45 reads: "**An isolated** retinoblastoma gene **DNA**

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[4] Exhibit 1004 consists of two documents – two page "Paper 2" and four page "Paper No. 14" – without unique page numbering. Therefore, pages 1-4 of "Paper No. 14" are referred to as pages 3-6, respectively, in this decision.

[5] Exhibit 1005 is a multi-document exhibit without unique numbering. The one page filing receipt is numbered as p. 1. The three page request for filing a divisional application is numbered as pp. 2-4. The five page preliminary amendment is numbered as pp. 5-9. The specification of the '290 application is numbered as pp. 10-53. The original claims are numbered as pp. 54-58. The abstract is numbered as p. 59. The twenty-three pages of drawings, which are not in consecutive figure number are numbered as pp. 60-82. Finally, the combined declaration and power of attorney are numbered as pp. 83-85. **Paragraph 20.1.1 of the STANDING ORDER (Paper 2) expressly instructs parties not to file an entire application file as a single exhibit**. When multiple documents are filed as a single exhibit, as with Exhibits 1004 and 1005, there are multiple pages with the same page number. In Exhibit 1005 there are at least five occurrences of "page 1."

molecule consisting of the nucleic acid molecule having the sequence shown in Figure 7" (Ex 1032, p. 3, emphasis added).

21. Figure 7 of the '947 application is said to be the nucleotide and predicted amino acid sequences of the retinoblastoma gene (Ex 1006, p. 36, ll. 7-8).

22. Dryja '290 application claim 26 depends from claims 25 and 1 and is directed to a "Purified nucleic acid comprising a human retinoblastoma gene, or a fragment thereof comprising 15 or more bases, said nucleic acid being less than 100 kb in size" (claim 1), "wherein the nucleic acid is a cDNA" (claim 25), and "wherein said **cDNA comprising a sequence of Figure 5**" (claim 26) (Ex 1032, p. 10, emphasis added).

23. **Figure 5** of the '290 application **is said to describe a nucleic acid sequence of a cDNA of the normal retinoblastoma gene**, with flanking regions (Ex 1005, p. 15, ll. 20-21, emphasis added).

24. The parties filed five joint miscellaneous motions for extensions of time for filing preliminary statements and preliminary motions (Exs 1007-1011), the last four of which expressly stated that the extension was being requested "to allow the parties to complete discussions in an attempt to settle the present interference".

25. On 3 August 1995, the Board issued an "ORDER" (Ex 1012) stating that

> ... The current motion for an extension of time represents the sixth [miscellaneous] motion for an extension of time for filing preliminary statements and preliminary motions sought by the parties. While the parties are encouraged to attempt to settle this interferences, the times set are for filing of preliminary statements and preliminary motions, not for conducting settlement negotiations.
>
> Accordingly, the time for filing preliminary statements and [preliminary] motions, if any, or, in lieu thereof, a settlement agreement

under 37 CFR 1.666 and a paper under 37 CFR 1.662(a), is reset to expire on **August 7, 1995**.

Any further request for an extension of time for filing preliminary statements and preliminary motions, sought for the purpose of negotiating a settlement will not be granted unless accompanied by a schedule, agreed to by the parties, establishing firm dates by which: (1) the settlement agreement will be executed; (2) proofs will be exchanged; (3) a determination as to priority will be made; and, (4) a paper under 37 CFR 1.662(a) will be filed.

Accordingly, any further request for an extension of time for the purpose of negotiating a settlement between the parties which does not comply with the requirements of the paragraph above will be treated as a waiver of the right of the parties to file any preliminary motions. [Italicized emphasis and bracketed material added.]

26. On 15 August 1995, the Board issued another "ORDER" (Ex 1013) stating that

(a) the joint [miscellaneous] motion for an extension of time filed 7 August 1995, based on its content, was being treated as the parties' notice of arbitration of interferences under 37 CFR 1.690(a);

(b) the notice was defective under 37 CFR § 1.690(a) because it failed to set the issues to be arbitrated, the name of the arbitrator or a date for the selection of the arbitrator, and to provide both that the arbitrator's award shall be binding on the parties and that the judgment thereon can be entered by the Board; and,

(c) "In light of all of the above, and in light of the undersigned's previous order (Paper Number 19 - mailed August 3, 1995), *the parties are considered to have waived their rights to file **any** preliminary motions*" (Ex 1013, p. 2, ¶ 2, italicized emphasis and bracketed material added).

27. The "ORDER" set 25 August 1995 and 15 September 1995 as the due dates for filing a notice complying with 37 CFR 1.690(a) and for filing preliminary

statements, respectively (Ex 1013, p. 2, last ¶).

28. On 17 October 1995, the Board issued still another "ORDER" (Ex 1014) stating:

> Receipt on August 25, 1995, of the paper captioned "JOINT MOTION FOR EXTENSION OF TIME" (Paper 22) is acknowledged. Therein, the parties represent that while the parties have signed an agreement to arbitrate the "issues relating to this interference, they do not intend to follow the formal provisions of 37 C.F.R. § 1.690." (page 1 of the motion). The parties further represent that in order to allow resolution of the issues as set forth in the schedule for arbitration on page 2 of the motion, an extension of time until October 31, 1995, is required. The motion is granted.
>
> However, the extended time is solely for the purpose of filing either a request for adverse judgment by the losing party in the arbitration under 37 CFR 1.662(a) or for the filing of preliminary statements by the parties. As noted by the undersigned in his order mailed August 15, 1995, *the parties have waived their right to file any preliminary motions in this interference* by pursing "informal" arbitration. [Ex 1014, italicized emphasis added.]

29. On 8 November 1995, the Board granted the parties an additional one week extension of time said to be necessary to allow the parties to complete the process of deciding priority, thereby retroactively extending the time for filing either a request for adverse judgment by the losing party or for filing preliminary statements to 7 November 1995 (Ex 1014).

30. The parties filed a number of papers on 7 November 1995, including Lee's request for adverse judgment (Ex 1017), a settlement agreement (Ex 1018), an arbitration agreement (Ex 1019), the arbitrator's decision on motions (Ex 1133) and Dryja's preliminary statement.[6]

31. On 2 January 1996, the Board issued an "ORDER TO SHOW CAUSE" which

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[6] Dryja's preliminary statement has not been made of record in this interference. However, receipt thereof by the Board in the '426 interference is acknowledged in Exhibit 1022, p. 1.

(a) noted that the last request for an extension of time was granted solely for the purpose of filing either preliminary statements or a request for adverse judgment, since both parties had waived their rights to file any preliminary motions by electing to pursue "informal" arbitration (Ex 1022, p. 2, ¶ 1);

(b) denied Lee's request to add claims 47-59 to its application because Lee had waived its right to file a preliminary motion (id., p. 3, ¶ 1);

(c) denied Lee's request for adverse judgment filed 7 November 1995 because Lee sought judgment against claims not involved in the '426 interference (id., p. 2, ¶ 2 - p. 4, ¶ 1); and,

(d) gave notice that judgment on the record would be entered against junior party Lee because Lee had failed to file a preliminary statement unless Lee showed cause why such action should not be taken (id., p. 4, ¶ 4).

32. In response to the Order to Show Cause (Ex 1022), Lee requested adverse judgment the '426 interference (Ex 1023).

33. On 31 January 1996, the Board issued a final judgment that "Wen-Hwa Lee and Eva Y-H.P. Lee, the junior party, are not entitled to a patent containing claims 15, 16, 45 and 46 of their application [07/951,947] corresponding to the Count" (Ex 1024, bracketed material added).

34. Thus, Lee lost the subject matter of application 07/951,947 claim 45 in the '426 interference, i.e., an isolated full-length, wild-type RB cDNA having the nucleotide sequence shown in Figure 7 (see FF 19 and 20, pp. 17-18 supra).

2. the subject matters of the '426 and the '182 interferences

35. The subject matter of the present '182 interference is essentially directed to a method of detecting a mutated RB gene nucleic acid by comparing a sample RB nucleic acid to the nucleic acid sequence of a normal RB gene using hybridization to detect the degree of sequence identity between the normal and

sample RB nucleic acid sequences.

In other words, the subject matter of the present '182 interference is essentially directed to a method of detecting a mutated RB nucleic acid by hybridizing a probe specific for a normal RB nucleic acid sequence, e.g., the isolated, full-length, wild-type RB cDNA having the nucleotide sequence shown in Lee '134 patent Figure 7, to a sample RB nucleic acid sequence to detect mutations, e.g., deletions, in the sample RB nucleic acid sequence vis-a-vis the probe sequence. (See FF 9-11, pp. 8-9 supra.)

36. The subject matter of the '426 interference was essentially directed to purified nucleic acids comprising a human retinoblastoma gene or a fragment[7] thereof, wherein the nucleic acid comprising 15 bases but less than 100 kb in size (see FF 18, p. 17 supra).

In other words, the generic subject matter of the '426 interference encompassed an obvious species, i.e., a purified full-length, wild-type RB cDNA, such as an isolated retinoblastoma gene DNA having the nucleotide sequence shown in Figure 7 of the Lee '947 application (see FF 20, pp. 17-18 supra), as well as other species including fragments of a human RB gene (see FF 18, p. 17 supra). Thus, the subject matter of the present interference is a method of using the obvious "species" of the subject matter of the '426 interference, namely using an isolated, purified full-length, wild-type RB cDNA, as a probe to detect abnormal or mutated RB nucleic acid in a mammalian

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[7] "Fragment" refers to a subsequence of nucleic acids within the human RB gene nucleic acid sequence, e.g., a restriction enzyme fragment thereof, with the proviso that the fragment is 15 to less than 100kb in size. A restriction enzyme recognizes a specific sequence in DNA and can cut at or near this sequence to produce fragments of the DNA. See e.g., Lee application '947 (Ex 1006), p. 13, l. 27 - p. 14, l. 3 and p. 19, l. 14 - p. 20, l. 1; and, Dryja application '290 (Ex 1005), p. 36, ll. 5-21.

cell sample.

3. the applications involved in the '426 and '182 interferences have essentially identical disclosures

37. The '627 application of Lee from which the '134 patent involved in the present interference issued (FF 2) is a continuation of the '947 application involved in the '426 interference (Ex 1142, front page).

38. The '158 application of Dryja involved in the present interference (FF 6) is a division of application 08/255,572, which is a continuation of application 07/951,342;[8] and, the '290 application of Dryja involved in the '426 interference is a division of application 07/951,342 (Ex 1005, p. 1).

Thus, the disclosures of the specifications of Lee involved in the '426 and '182 interferences and the disclosures of the specifications of Dryja involved in the '426 and '182 interferences are essentially identical.

4. the applications involved in the '426 interference disclose methods for detecting abnormal or mutated retinoblastoma genes

39. According to Lee's '947 specification, the "invention relates to the cloning, isolation, identification and sequencing of the retinoblastoma gene" and "to the method of use of the cloned retinoblastoma gene cDNA as a tool for diagnosing retinoblastoma" (Ex 1006, p. 1, ll. 10-14).

40. Further according to the '947 specification,

> There are two approaches to utilize the current invention as a

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[8] See Paper 39, p. 3 where Lee admits Dryja Statement of Material Fact ("SMF") number 1 as set forth in Paper 33, ¶ bridging pp. 1-2.

> diagnostic tool for diagnosing retinoblastomas. The first one is to use the RB cDNA or genomic DNA as probes to determine the defect region of the mutated RB gene through genomic DNA blotting analysis or using the method of restriction fragment length polymorphism analysis[9] to determine the diseased allele. [Ex 1006, ¶ bridging pp. 27-28, fn. added.]

41. Moreover, original claim 23 of the '947 application read (Ex 1006, p. 66):

> A method of diagnosing tumorigenesis in a mammal comprising isolating from the cells of said mammal an appropriate fraction normally containing its mRNA transcript; attempting to hybridize said fraction with an appropriate cloned RB cDNA probe, said cloned RB cDNA probe being produced from a cloned normal RB gene of a similar mammal; and determining whether said mRNA transcript is present in said fraction or whether an abnormal mRNA transcript was present or whether a normal mRNA transcript was present, whereby the absence of mRNA or the presence of abnormal mRNA transcript indicates tumorigenesis.

42. Similarly, according to Dryja's '290 specification, "the invention concerns purified nucleic acid (less than 100kb in size), and fragments thereof of at least 15 bases, encoding the Rb gene" (Ex 1005, sentence bridging pp. 11-12).

43. Further according to the '290 specification, "[t]he cDNA and genomic sequences, e.g., those in p4.7R, can be used, according to the invention, to screen individuals for the presence of a mutated allele of the Rb gene" (Ex 1005, p. 25, ll. 23-25).

44. Original claim 10 of Dryja's '290 application is directed to a method of detecting large deletions in the RB gene of a human patient comprising hybridizing a nucleic acid sample from the patient with p4.7R cDNA, or a fragment thereof,

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[9] Restriction fragment length polymorphism analysis is a Southern blot analysis of DNA that has been digested with restriction enzymes and hybridized with cloned probes to demonstrate differences in the length of restriction fragments. These variations in length are due to mutations, which cause either the loss or the gain of a restriction site, or more rarely, insertions or deletions within a restriction fragment. See e.g., Darnell et al., MOLECULAR CELL BIOLOGY, second edition, Scientific American Books, Inc., distributed by W.H. Freeman and Company, New York, NY, pp. 209-210 and 325-326 (copy enclosed).

wherein lack of hybridization indicates the presence of a large deletion in the patient's gene.[10]

45. According to the Dryja '290 specification, p4.7R contains the nucleotide sequence of the normal retinoblastoma gene (Ex 1005, p. 31, ll. 14-15).

To recap, the disclosures of the Lee '610 patent specification involved in the present interference and the Lee '947 application specification involved in the '426 interference are essentially identical (FF 2, 16 and 38, pp. 7, 17 and 23 supra). The disclosures of the Dryja '158 application specification involved in the present interference and of the Dryja '290 application specification involved in the '426 interference are essentially identical (FF 6, 17 and 38, pp. 7-8, 17 and 23 supra). Both parties' specifications are said to describe an isolated full-length, wild-type RB cDNA,

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[10] Original claims 8-10 in Dryja's '290 application read (Ex 1005, pp. 54-55):

8. A method of detecting large deletions in the retinoblastoma gene of a human patient predisposing said patient to retinoblastoma, comprising the steps of:
hybridizing a nucleic acid sample from said patient with a probe specific for the retinoblastoma gene, and
determining the ability of said probe to hybridize to said nucleic acid,
wherein lack of hybridization to said nucleic acid indicates the presence of a large deletion in said gene.

9. A method of detecting large deletions in the retinoblastoma gene of a human patient that may predisposing [sic] said patient to retinoblastoma, comprising the steps of:
generating nucleic acid fragments from a sample of said patient;
separating said fragments according to a determined physical property of said fragments,
hybridizing a probe specific for the retinoblastoma gene to said fragments,
detecting hybrids of said probe and said fragments, and
comparing said hybrids to hybrids detected from the hybridization of said probe and separated nucleic acid fragments from a normal retinoblastoma gene,
wherein the absence of hybrids, or the smaller size of said hybrids from the sample of said patient is an indication of large deletions in the retinoblastoma gene of said patient.

10. The method of claim 8 or 9, wherein the probe specific for the retinoblastoma gene is the cloned DNA in p4.7R, or a fragment thereof.

i.e., the nucleic acid of Lee '947 Figure 7 and the nucleic acid of Dryja '290 Figure 5 (FF 21 and 23, p. 18 supra). Lee '947 application claim 45, directed to an isolated retinoblastoma DNA consisting of a nucleic acid molecule having the sequence shown in Figure 7, corresponded to the sole count of the '426 interference (FF 19 and 20, pp. 17-18 supra). Dryja '290 application claim 26, directed to a purified nucleic acid comprising a cDNA comprising a sequence of Figure 5, corresponded to the sole count of the '426 interference (FF 19 and 22). Both Lee's '947 specification and Dryja's '290 specification expressly state their invention includes using RB cDNA as a tool for diagnosing retinoblastoma and suggest using RB cDNA or fragments thereof to detect abnormal or mutated RB nucleic acids (FF 39-40 and 43, pp. 23-24 supra). Both the '947 and '290 applications involved in the '426 application contained original claims directed to using RB gene cDNA or fragments thereof as hybridization probes to detect mutated Rb genes or nucleic acids (FF 41 and 44, pp. 24-25 supra). Finally, Lee requested and received adverse judgment in the '426 interference (FF 32 and 33, p. 21 supra). Thus, Lee lost the subject matter of Lee application '947 claim 45, i.e., an isolated full-length, wild-type RB cDNA having the nucleotide sequence shown in Figure 7, in the '426 interference (FF 34, p. 21 supra).

5. discussion

Because Lee's '947 application and Dryja's '290 application involved in the '426 interference, respectively, have essentially the same disclosure as Lee's '134 patent and Dryja's '158 application involved in the present interference (based on divisionals and continuations), the priority issue of the subject matter of the present interference

could have been raised and determined in the '426 interference. Indeed, the Lee and Dryja applications involved in the '426 interference not only stated that the subject matter of the present interference was an express aspect of the invention disclosed in their respective specification, but they also contained original claims directed to ths subject matter. Either party could have filed a Rule 633(c) motion in the '426 interference to redefine the interfering subject matter. For example, either party could have moved under Rule 633(c)(1) to add a count directed to a method of detecting an abnormal or mutated retinoblastoma nucleic acid (RNA or DNA) by hybridizing an isolated, full-length, wild-type RB cDNA probe, to add appropriate method of use claims to its application and to its opponent's application and to designate the added claims as corresponding to the added count in the '426 interference. Alternatively, either party could have moved under Rule 633(c)(2) and (c)(3) to add appropriate method of use claims to its application and to its opponent's application and to designate the added claims as corresponding to the count in the '426 interference.

Under estoppel § 41.127(a)(1) (former Rule 658(c)), a judgment in an interference settles, among other things, all issues which could have been properly raised and decided by an additional count. Here, the issue of priority of invention of a method of detecting an abnormal or mutated retinoblastoma nucleic acid (RNA or DNA) by hybridizing an isolated, full-length, wild-type RB cDNA probe could have been raised and decided if Lee had filed the proper preliminary motions during the '426 interference. Under § 41.127(a)(1), a losing party who could have properly moved for relief on an issue, but did not so move, may not take action in the Office after the judgment that is

inconsistent with that party's failure to move.

According to Dryja, (i) an isolated, full-length wild-type RB cDNA is part of the subject matter on which judgment against Lee was awarded (Paper 33, pp. 17-18), (ii) using an isolated, full-length wild-type RB cDNA as a hybridization probe to detect RB nucleic acid (gene) mutations is the precise utility described in the Dryja applications (id., p. 19), and, therefore, (iii) the decision of the arbitrator and the judgment of the Board in the '426 interference awarded Dryja the subject matter of the claims of Lee's '134 patent (id., p. 22). Specifically, Dryja argues that

> Lee was aware that the Dryja application in the '426 interference supported claims for methods of hybridization using the nucleic acid sequence for the Rb protein. Indeed, Dryja's preferred utility was based on methods of hybridization of the nucleic acid sequence for the Rb protein, ... **Lee's failure to add a count** during interference, when he could have and should have (to resolve all issues between the parties), precludes him from raising the subject matter again post-interference. ... [Id., p. 24, ¶ 2, emphasis added.]

The Notice of Final Rule, Patent Interference Proceedings, 49 Fed. Reg. at 48426-27, 1050 O.G. at 395, provided several examples of how estoppel under Rule 658(c) (now § 41.127(a)(1)) applies. In Example 25, junior party applicant AL and senior party applicant AK both disclosed separate patentable inventions "A" and "B" and claimed only invention "A" in their respective applications. An interference was declared with a single count to invention "A". Neither party filed a preliminary motion under Rule 633(c)(1) (now § 41.121(a)(i)) to add a count to invention "B". Judgment as to all of AK's claims corresponding to the sole count was awarded to senior party applicant AK. Junior party applicant AL is estopped to obtain a patent containing claims to invention "B". Senior party applicant is not estopped to obtain a patent containing

claims to invention "B".

In interference '426, junior party applicant Lee (07/951,947) and senior party applicant Dryja (07/958,290) both disclosed invention "A" (i.e., purified nucleic acids comprising a human retinoblastoma gene or a fragment thereof, wherein the nucleic acid comprised 15 bases but was less than 100 kb in size, which encompasses a purified full length, wild-type RB cDNA) and invention "B" (i.e., a method of detecting an abnormal or mutated retinoblastoma nucleic acid (RNA or DNA) by hybridizing an isolated, full length, wild-type RB cDNA probe). At the time the '426 interference was declared with a single count to invention "A", the parties apparently only had pending claims to invention "A". Both parties waived their right to file any preliminary motions by failing to act timely. Judgment as to all of junior party's claims corresponding to the sole count was awarded against Lee. Junior party Lee, the losing party, is estopped from obtaining a patent containing claims to invention "B". Senior party Dryja is not estopped from obtaining a patent containing claims to invention "B".[11]

Applying estoppel under § 41.127(a)(1), and former Rule 658(c), in this case is consistent with the purpose of the rule, i.e., to resolve as many priority issues between the parties as possible in a single inter partes proceeding. As discussed above, the rule

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[11] In contrast, a preliminary motion under former Rule 633(e) to declare an additional interference was interpreted to require that at least one party claim the additional invention. For example, estoppel would apply if (1) a party's non-involved application claimed the invention which should have been the subject of a second interference and its opponent's application claimed that invention; (2) a party's non-involved application disclosed the invention which should have been the subject of a second interference and its opponent's application claimed that invention; or, (3) a party's non-involved application claimed the invention that should have been the subject of a second interference and its opponent's involved application disclosed that invention. Estoppel would not apply if (4) a party's non-involved application disclosed the invention which should have been the subject of the second interference and its opponent's involved application disclosed that invention. See 50 Fed. Reg. 23122, 23123 (May 31, 1985), reprinted in 1059 O.G. 27, 37 (Oct. 22, 1985).

does not require the subject matter to be claimed at the time the prior interference is declared in order for it to be the basis of an estoppel.

We are mindful that "[e]stoppel should be decided on the facts of each case with reference to principles of equity." In re Kroekel, 803 F.2d 705, 709, 231 USPQ 640, 643 (Fed. Cir. 1986). Here, there is no reason not to apply the estoppel rule as written.

Lee argues that Dryja's motion is procedurally defective because it does not include a comparative claim chart (Paper 39, pp. 13-17). Lee contends that "[w]ithout claim charts or resort to the alternative format expressly authorized by the Standing Order, the comparison urged by Dryja Motion is needlessly confusing and improperly attempts to shift the burden to the Board and its opponent to perform the exercise" (id., p. 16, ll. 2-4). Lee also points out that failure to comply with the Board's Rules and Standing Order may result in dismissal of the motion, citing LeVeen v. Edwards, 57 USPQ2d 1406 (Bd. Pat. App. & Int. 2000).

First, while Dryja Revised Motion 2 is hardly a paradigm of procedural perfection, it does nonetheless place the estoppel question fairly at issue for reasons discussed above. Second, the level of procedural error here does not rise to the level of error found in LeVeen. In LeVeen, Edwards filed a Rule 633(a) motion that certain LeVeen claims were anticipated under 35 U.S.C. § 102(e) over U.S. Patent 5,458,597 ("the '597 patent") or obvious under 35 U.S.C. § 103 over the '597 patent in view of other prior art. The Board, exercising its discretion, dismissed Edwards' motion because (1) it was not presented in a procedurally proper fashion and (2) it did not state a claim upon which relief may be granted. Although Edwards had actual notice of

interference procedural rules, its motion incorporated "arguments" by reference to declarations. The motion failed to set forth all relevant facts with reference to the evidence in support of the facts, failed to present an argument as to why the facts justify the relief requested in the motion, and failed to use the required format. The list of exhibits relied upon as set forth in Edwards' motion did not include two exhibits listed in the declarations or the declarants' C.V.s. Moreover, Edwards' anticipation argument failed to explain where the '597 patent described every limitation required by LeVeen's claims at issue. Edwards' obviousness argument similarly failed to identify the differences between the prior art and the claims and to provide a reason, suggestion or motivation in the prior art that would lead one of ordinary skill in the art to combine the elements of the prior art to make the specific combination claimed by LeVeen and to suggest a reasonable likelihood of success.

Here, Lee responded to each of Dryja's alleged facts in its statement of material facts (SMF) and arguments. Although Dryja's motion failed to include a claim chart or separate listing of the "Evidence Relied Upon", Dryja's SMFs did discuss the relationship between Lee's '134 patent claims and the disclosures of the applications involved in the '426 interference as well as provide parenthetical references to the exhibits being relied upon in support of its arguments. Dryja's motion presented arguments as to why its asserted facts justified the relief requested in the motion. Lee does not complain that it was unable to determine Dryja's position or that Dryja's motion caused it any actual prejudice. Rather, Lee Opposition 2 (Paper 39) contains both arguments and supporting evidence in rebuttal to Dryja's position.

While Dryja's motion may have been somewhat sloppy and would have been clearer had Dryja provided comparative claim charts and followed all other procedural requirements, Lee does not appear to have suffered any significant prejudice. Therefore, we exercise our discretion to decline to dismiss Dryja Revised Motion No. 2 on purely procedural grounds given the importance of interference estoppel in securing "the just, speedy, and inexpensive resolution of every proceeding before the Board." 37 CFR § 41.1(b). To do otherwise under the particular circumstances of this interference would be to elevate form over substance and to distort the policy behind estoppel. As stated in Shimer, 69 F.2d at 558, 21 USPQ at 163, "[i]f the rule be not enforced or enforceable, then delays and litigation are greatly increased. It is quite obvious that the doctrine of estoppel, as applied in these cases, results in the better conduct of the business of the Patent Office and in the public good." This is particularly true here where the present interference is based on an application and a patent with essentially the same disclosures as the applications involved in the '426 interference (based on continuations and divisionals) and where the subject matter of this interference was fairly described as part of "the invention" in the specifications of the applications involved in the '426 interference. Estoppel is an equitable doctrine to be decided on the facts of each case. In this particular case on these particular facts, the balance of equities favors application of the doctrine. Lee was fully aware of Dryja's inventive disclosure and, so advised, it became Lee's duty to place in issue every priority issue that it wished to contest. Rule 633 afforded Lee this opportunity in the '426 interference. Lee did not avail itself of the afforded opportunity in the '426 interference

and will not now be permitted to evade the consequences of its inaction solely on the basis of a procedural defect in Dryja's motion.

Lee argues that it was not afforded the opportunity to file preliminary motions in the '426 interference because "[b]oth parties waived their rights to file any preliminary motions while they sought, in joint motions, extensions of time to settle that interference" (Paper 39, p. 19, ¶ 2). However, Lee knowingly and voluntarily decided to waive its right to file any preliminary motions in the '426 interference by continuing to seek the extensions of time.

When the '426 interference was declared, 1 May 1995 was set as the time for filing preliminary motions and preliminary statements (FF 15, pp. 16-17 supra). On 3 August 1995, after receiving a fifth request for an extension of time for filing preliminary statements and preliminary motions in order to allow the parties to negotiate a settlement agreement, the Board advised the parties that, while settlements are encouraged, the time set for filing preliminary motions and preliminary statements, not for conducting settlement negotiations. The time for filing preliminary motions and preliminary statements, or in lieu thereof, a settlement agreement (37 CFR § 1.666) and request for entry of adverse judgment (37 CFR §1.662(a)), was extended for a fifth time to 7 August 1995. The parties were explicitly warned that any further request for an extension of time for filing preliminary motions and preliminary statements for the purpose of negotiating a settlement agreement between the parties which did not comport with Rules 666 and 662(a), would be treated as a waiver of the parties' right to file any preliminary motion. [FF 24-25, pp. 18-19 supra.] A joint motion for an extension

of time was filed 7 August 1995 which, based on its content, was treated as the parties' notice of arbitration of interferences under 37 CFR § 1.690(a), and the parties were deemed to have waived their rights to file any preliminary motions (FF 26, p. 19 supra). Lee clearly had at least six opportunities between 1 May 1995 and 7 August 1995 to file preliminary motions in the '426 interference and also had ample notice of the consequences of failing to do so. Moreover, Lee was expressly advised that involvement in settlement negotiations does not excuse a party from meeting deadlines set by the Board.

In general, the settlement of interferences is encouraged. Cf. 35 U.S.C. § 135(d) (arbitration). However, the failure to take appropriate action during an interference proceeding cannot ordinarily be excused on the basis that the defaulting party was engaged with its opponent in settlement negotiations. See Tsuruta v. Nardella, 60 USPQ2d 1822, 1825-26 (Bd. Pat. App. & Int. 2001) and 4 Revise and Caesar, INTERFERENCE LAW AND PRACTICE, §§ 861 and 864, pp. 2956 and 2963-63 (The Michie Company, Charlottesville, VA 1948). According to the Notice of Final Rule, Arbitration of Patent Interference Cases, 52 Fed. Reg. 13833, 13834 (Apr. 27, 1987), 1080 Off. Gaz. Pat. Off. 15 (May 16, 1987) (bracketing added),

> the Commissioner's [now Director's] Notice of Nov. 9, 1976, titled "Extensions of Time and Filing of Papers in Interferences," 953 Official Gazette 2 (Dec. 7, 1976) ... [stated] that:
>
> > * * * stipulation or motions for extensions of time under 37 CFR 1.245 will not henceforth be approved or granted, respectively, unless accompanied by a detailed showing of facts sufficient to establish that the action for which the extension is sought could not have been or cannot be taken or completed during the time previously set therefor, and

> that the entire extension appears necessary for the taking or completion of that action. Since the Office favors the amicable settlement of interferences, the foregoing requirement will be liberally applied in the case of a first request for extension of time for the purpose of negotiating settlement.
>
> Consequently, the examiner-in-chief [now Administrative Patent Judge, "APJ"] may give favorable consideration to a motion for an extension of time for purposes of settlement; however, a further motion for an extension for that purpose would not be granted unless it is accompanied by a schedule of specific dates showing that the parties will make a good faith effort to promptly terminate the proceeding. If preliminary motions under 37 CFR 1.633 have not been filed, the examiner-in-chief would not normally extend the time for their filing merely for purposes of settlement. In these circumstances, the examiner-in-chief would require that the preliminary motions be filed or that their filing be waived.

Here, the APJ granted multiple extensions of time for purposes of settlement negotiations. The time for filing preliminary statements and preliminary motions was set for a sixth time to 7 August 1995. The parties were advised that if they chose to file a settlement agreement under 37 CFR § 1.666 and a request for adverse judgment under 37 CFR § 1.662(a) instead of filing preliminary statements and motions, any further request for an extension of time must be accompanied by a schedule establishing firm dates by which (1) the settlement agreement would be executed, (2) proof would be exchanged, (3) a determination as to priority would be made, and (4) a paper under Rule 662(a) would be filed or the request would not be granted. The parties were warned expressly that if the time schedule did not accompany the settlement agreement, any further request for an extension of time for the purpose of negotiating a settlement would be treated as a waiver of their right to file any preliminary motions. Yet the parties asked for and received three additional extensions of time to 25 August

1995, 31 October 1995 and 7 November 1995, albeit after having waived their right to file any preliminary motions. [See FF 24-29, pp. 18-20, supra.]

Continued extensions of time for some nebulous reason is inconsistent with the efficient administration of justice in interference cases. "Patent interferences shall be administered such that pendency before the Board is normally no more than two years." 37 CFR § 41.200(c) (former Rule 610(c)). The Board, as a tribunal, must be in control of its own docket, case management and workflow. If parties were routinely excused from meeting deadlines set by the Board, the result would be uncertainty and unnecessary delay. The following appeared in the Notice of Final Rule, Patent Interference Proceedings, 49 Fed. Reg. at 48445, 1050 O.G. at 414:

> When counsel and an examiner-in-chief agree to a schedule and times are set, the parties will be expected to adhere to the schedule unless there are unusual circumstances. Apart from work that counsel may have in an interference, an examiner-in-chief will have a docket and must manage not only the interference involving counsel, but numerous other interferences. The U.S. Court of Appeals for the Federal Circuit recently said the following in *Rosemount, Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1549-1550, 221 USPQ 1, 10 (Fed. Cir. 1984):
>
> > The conduct of a trial, granting of continuances and the like, is not, however, solely or entirely a matter of balancing conveniences of the parties. The Federal Rules of Civil Procedure recognize another consideration – the need for the exercise of discretion by the trial court in carrying out its duty of managing the judicial process, the business of the court, and the administration of justice.
>
> Likewise, these rules also recognize the need for the exercise of discretion by an examiner-in-chief in carrying out his or her duty of managing the interference (§ 1.610), the business of the PTO (§ 1.610), and the administration of justice (§ 1.601).

Lee cannot deny that it was afforded at least six different opportunities to file preliminary motions in the '426 interference up through 7 August 1995. Lee cannot

deny that the Board acted within its discretion by giving the parties a choice in its "ORDER" of 3 August 1995 (Ex 1012) of filing preliminary statements and motions, if any, or, in lieu thereof, a settlement agreement under 37 CFR 1.666 and a paper under 37 CFR 1.662(a). Lee cannot deny that the Board warned both parties that any further request for an extension of time for the purpose of negotiating a settlement that did not comply with the terms set forth in its 3 August 1995 "ORDER" (Ex 1012) would be treated as waiver of the parties' right to file any preliminary motions. Lee cannot deny that the Board explicitly advised the parties that the times set during the motions phase of the interference are for filing preliminary statements and motions, not for conducting settlement negotiations. Settlement negotiations might break down unexpectedly. In short, settlement negotiations notwithstanding, nothing prevented either party from filing preliminary motions by 7 August 1995.

Thus, based on the foregoing, Lee knowingly and voluntarily waived its right to file any preliminary motions in the '426 interference. Lee's argument to the contrary is without merit.

6. interference proceedings and arbitration

Interferences are provided for by 35 U.S.C. § 135(a), which reads in relevant part

> Whenever an application is made for a patent which, in the opinion of the Director, would interfere with any pending application, or with any unexpired patent, an interference may be declared and the Director shall give notice of such a declaration to the applicants, or applicant and patentee, as the case may be. The Board of Patent Appeals and Interferences shall determine questions of priority of the inventions and may determine questions of patentability. Any final decision, if adverse to the claim of the applicant, shall constitute the final refusal by the Patent and Trademark Office of the claims involved, and the Director may issue a patent to the applicant who is adjudged the prior inventor. ...

Once the interference is declared, the Board acquires exclusive jurisdiction over any involved file. 37 CFR § 41.103 (old Rule 614). Once declared, the interference proceeds in two phases. The first phase, or "motion" phase (formerly "preliminary motion" phase), defines and focuses the subject matter of the interference. As discussed above, parties may file motions to change the scope of the interference by changing, adding or deleting counts or by changing the designation of claims as corresponding or not corresponding to the count, etc. The decision on motions determines the ultimate subject matter of the interference. The second or "priority" phase of the interference determines which party was the first inventor of the interfering subject matter.

Lee implicitly argues that by raising a motion to add or substitute a count during arbitration it had effectively raised the issue of the subject matter of this interference in the '426 interference proceeding. In its opposition (Paper 39, p. 19, ¶ 3), Lee argued that (emphasis added)

> Despite not being able to file motions in the interference, Lee did file a motion in the arbitration, which was made of record in the interference, that raised the issue of separate patentability of full length human retinoblastoma gene. See, Exhibit 1032, "Lee Motion (3) Pursuant to 37 C.F.R. § 1.633(c)(1) to Add or Substitute a Count." See Lee Fact ¶8. In that paper, Lee set forth the basis for its belief that the full length normal wild-type human retinoblastoma gene defined an invention separately patentable from a count drawn to fragments of the gene. Although the arbitrator did not agree with Lee's position, Lee's motion raised the issue of the full length nucleic acid claims, and Dryja was on notice that Lee believed that it was separately patentable. Lee made this arbitration motion officially of record in the interference, along with the Arbitrator's Decision. Dryja was on notice of the issue, whether through the arbitration, or through the record made before the Board. Lee Fact ¶8.

Lee provides no legal basis in support of its position. Moreover, to the extent the arbitration is relevant at all, it is equivalent to Lee's motion having been denied. Lee acquiesced in the result by filing a request for adverse judgment.

While arbitration pursuant to 35 U.S.C. § 135(d)[12] and 37 CFR § 41.126[13] (former

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[12] According to 35 U.S.C. § 135(d),

Parties to a patent interference, within such time as may be specified by the Director by regulation, may determine such contest or any aspect thereof by arbitration. Such arbitration shall be governed by the provisions of title 9 to the extent such title is not inconsistent with this section. The parties shall give notice of any arbitration award to the Director, and such award shall, as between the parties to the arbitration, be dispositive of the issues to which it relates. The arbitration award shall be unenforceable until such notice is given. Nothing in this subsection shall preclude the Director from determining patentability of the invention involved in the interference.

[13] According to 37 CFR § 41.126,

(a) Parties to a contested case may resort to binding arbitration to determine any issue in a contested case. The Office is not a party to the arbitration. The Board is not bound and may independently determine questions of patentability, jurisdiction, and Office practice.

(b) The Board will not authorize arbitration unless

(1) It is to be conducted according to Title 9 of the United States Code.

(2) The parties notify the Board in writing of their intention to arbitrate.

(3) The agreement to arbitrate:

(i) Is in writing,

(ii) Specifies the issues to be arbitrated,

(iii) Names the arbitrator, or provides a date not more than 30 days after the execution of the agreement for the selection of the arbitrator, and

(iv) Provides that the arbitrator's award shall be binding on the parties and the judgment thereon can be entered by the Board.

(4) A copy of the agreement is filed within 20 days after its execution.

(5) The arbitration is completed within the time the Board sets.

(c) The parties are solely responsible for the selection of the arbitrator and the conduct of the proceedings before the arbitrator.

(d) Issues not disposed of by the arbitration will be resolved in accordance with the procedures established in this subpart.

(e) The Board will not consider the arbitration award unless it:

(1) Is binding on the parties,

(2) Is in writing,

(3) States in a clear and definite manner each issue arbitrated and the disposition of each issue, and

(4) Is filed within 20 days of the date of the award.

(f) Once the award is filed, the parties to the award may not take actions inconsistent with the award. If the award is dispositive of the contested subject matter for a party, the Board may enter judgment as to that party.

Rule 690) is officially encouraged, the Office is not a party to the arbitration. However, the parties voluntarily and knowingly not only waived their right to file any preliminary motions, including motions to add or substitute a count, but also elected to pursue "informal" arbitration (FF 25-29, pp. 18-20 supra). Both parties must live with the consequences of their conscious litigation decision. Lee requested and received adverse judgment in the '426 interference. Now Lee must live with the consequences of its actions.

Simply put, Lee never properly raised before the Board the issue of whether an isolated, purified nucleic acid encoding a full length, wild-type RB protein was a separately patentable invention from the subject matter of the sole count in the '426 interference, i.e., a purified nucleic acid comprising a human RB gene or a fragment thereof, wherein the nucleic acid comprised at least 15 bases but less that 100 kb in size. Furthermore, to the extent Lee did raise the issue during arbitration in Lee Motion 3, it is noted that the motion was denied by the arbitrator because, in the arbitrator's opinion, Lee had not carried its burden as movant (Ex 1133, ¶ bridging pp. 6-7).

Finally, we also note that the arbitrator appeared to be of the opinion "that Dryja could not support any claims to be designated as corresponding to proposed substitute count 1" as set forth in Lee's arbitration motion 3 (id.). Proposed substitute count 1, according to Lee's motion, was directed to a purified nucleic acid comprising a normal (wild-type) human RB gene encoding a full length, normal (wild-type) human RB protein, said protein being less than 100 kb in size (Ex 1032, ¶ bridging pp. 2-3). Since the disclosures of the specifications of Dryja applications '290 and '158 involved in the

'426 and present '182 interferences, respectively, are essentially identical (FF 38, p. 23 supra), there is an implication that the specification of the Dryja '158 application involved in the present interference likewise could not support a claim directed to subject matter comprising a purified nucleic acid comprising a human RB gene encoding a full length, wild-type human RB protein, e.g., an isolated full length, wild-type RB cDNA. Lee has not, however, moved for judgment that Dryja's claims involved in the present '182 interference, i.e., method claims 22, 23 and 49, which all require use of an isolated full length, wild-type RB cDNA, are unpatentable for failing to satisfy the written description requirement of 35 U.S.C. § 112, first paragraph.

First, the Board is not bound by the arbitrator's decision on, and may independently determine, questions of patentability. 37 CFR § 41.126(a) (formerly Rule 690(d)). Therefore, the Board is not bound by the '426 interference arbitrator's opinion that the Dryja '290 application could not support a claim directed to subject matter comprising a purified nucleic acid comprising a human RB gene encoding a full length, wild-type human RB protein, e.g., an isolated full length, wild-type RB cDNA.

Second, unpatentability for lack of written descriptive support under § 112, first paragraph, is expressly identified in the current interference rules as a threshold issue which, if resolved in favor of the movant, deprives the opponent of standing in the interference. 37 CFR § 41.201. Thus, had Lee successfully moved that involved Dryja claims 22, 23 and 49 are unpatentable for lack of written descriptive support under the first paragraph of § 112, Dryja revised motion 2 would have been moot. Despite the express invitation in the rule, Lee did not seek this relief.

To the extent Lee's motions could be parsed to request such relief, we decline to make out a party's case for it. Ernst Haas Studio, Inc. v. Palm Press, Inc., 164 F.3d 110, 112, 49 USPQ2d 1377, 1379 (2d. Cir. 1999). In any case, we reviewed both of Lee's remaining motions, Motions 1 and 2 (Papers 48 and 49, respectively), filed in this interference to see if Lee had moved that Dryja's involved method claims are unpatentable for failure to satisfy the description requirement of § 112, first paragraph.

46. In both Lee Motions 1 and 2, Lee explicitly states as a material fact in support of its motion that "[t]he earliest date on which Dryja filed an application disclosing a full-length wild-type RB cDNA is January 23, 1989, the date of filing for U.S. Patent Application No. 07/300,667 ('the '667 application')" (Paper 48, p. 33, Lee SMF 33; Paper 49, p. 4, Lee SMF 19).

The Dryja '158 application involved in the present interference is a division of application 08/255,572, which is a continuation of application 07/951,342 (FF 38, p. 23 supra).

47. Dryja application 07/951,342 is a continuation of Dryja application 07/728,756, which is a continuation of Dryja application 07/300,667 (Ex 1005, p. 1).

Thus, the disclosures of the specifications of Dryja's '158 and '667 applications are essentially identical. Consequently, it follows that the specification of the Dryja '158 application involved in the present interference likewise discloses a full length, wild-type RB cDNA based on Lee's explicitly statement of material fact that the specification of

the '667 application discloses a full length, wild-type RB cDNA.[14] Therefore, Lee not only failed to move that involved Dryja claims 22, 23 and 49 are unpatentable for lack of written descriptive support under the first paragraph of § 112 in the present interference, but also evidenced statements to the contrary. Thus, it would be manifestly unfair to Dryja if the Board were to make out a case for Lee that Dryja's involved claims were unpatentable for lack of written descriptive support under § 112, first paragraph.

In short, neither Dryja, the USPTO nor the public should be penalized for Lee's failure to avail itself of the opportunities afforded it under the interference rules.

D. the '259 interference

48. There is no dispute that the subject matter of the '259 interference encompassed antibodies which specifically bind various RB proteins or fragments thereof and immunoassay methods using said antibodies to determine the presence or absence of a normal RB protein in a sample in order to diagnosis complete or partial inactivation of the RB gene.[15]

Based upon our conclusion above that Lee '134 patent claims 1-4 are barred by the doctrine of estoppel based on a failure to move in the '426 interference, it is unnecessary for us to consider whether the '259 interference provides a separate and

---

[14] Moreover, since the Dryja '290 application involved in the '426 interference is a division of the same Dryja 07/951,342 application, the disclosures of the specifications of Dryja's '290 and '667 applications are also essentially identical. Consequently, it also follows that the specification of the Dryja '290 application involved in the '426 interference likewise discloses a full length, wild-type RB cDNA based on Lee's explicitly statement of material fact that the specification of the '667 application discloses a full length, wild-type RB cDNA.

[15] See Paper 39, p. 5 where Lee admits Dryja SMF 27 as set forth in Paper 33, p. 7.

independent basis for interference estoppel. We take no position on whether the subject matter of the '259 interference is directed to the same patentable invention as the subject matter of the present '182 interference.

E. Miscellaneous comments

Interference 105,182 is related to interference 105,264. The subject matter of interference 105,264 is directed to isolated and purified Rb gene product proteins with a molecular weight of 110 to 114 KD. As noted by Lee, Dryja has not filed a motion alleging estoppel in the '264 interference (Paper 39, p. 21, ¶ 2). Thus, we take no position in this interference on whether or not the '259 interference might provide a basis for interference estoppel in the related 105,264 interference.

V. Lee Motions 1 and 2 and Dryja Motion 4

"The Board may take up motions for decision in any order, may grant, deny, or dismiss any motion, and may take such other action appropriate to secure the just, speedy, and inexpensive determination of the proceeding." 37 CFR § 41.125(a). Since our decision on Dryja revised motion 2 is dispositive, the remaining motions, i.e., Lee motions 1 and 2 and Dryja motion 4, are **dismissed** as moot.

VI. Order

Based on the foregoing and for the reasons given, it is

ORDERED that Dryja revised motion 2 (interference estoppel) is **granted**;

FURTHER ORDERED that Lee motion 1 (attacking benefit), Lee motion 2 (unpatentability over prior art) and Dryja motion 4 (unpatentability over prior art) are **dismissed** as moot in view of the grant of Dryja revised motion 2.

\ Richard Torczon \ )
RICHARD TORCZON)
Administrative Patent Judge)
)
)
\ Carol A. Spiegel \ ) BOARD OF PATENT
CAROL A. SPIEGEL) APPEALS AND
Administrative Patent Judge) INTERFERENCES
)
)
\ Sally Gardner Lane \ )
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Administrative Patent Judge)

Enc.: MOLECULAR BIOLOGY AND BIOTECHNOLOGY: A COMPREHENSIVE DESK REFERENCE, R. Meyers, ed., VHC Publishers, Inc., New York, NY (1995), pp. 428-431 and 817-820.

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HUMAN GENETIC PREDISPOSITION TO DISEASE

Belinda J. F. Rossiter and C. Thomas Caskey

Key Words

Codon Three adjacent nucleotides in messenger RNA that specify which amino acid is to be placed in a protein during translation.

DNA Polymorphism Variation in DNA sequence.

Exon Portion of a gene that is present in mRNA after RNA processing.

Gene Portion of DNA that is required for production of a functional product.

Genome Sum of the genetic information contained in one representative of each chromosome pair.

Intron Portion of a gene that is excised during RNA processing.

Mutation Alteration in a gene.

Oncogene Gene that results in uncontrolled cell growth when activated by mutation.

Phenotype Observable characteristics of an organism.

Promoter DNA sequences controlling the expression of a gene.

Stop Codon Triplet nucleotide in mRNA that signals termination of protein synthesis.

Tumor Suppressor Gene Gene that normally acts to regulate cell growth, whose inactivation by mutation leads to uncontrolled cell growth.

The human genome is very complex, and alterations causing genetic diseases can occur in a variety of ways. As genetic research advances, more rapid and accurate diagnosis of genetic disease is possible, and genetic methods can even be used as a form of therapy. Some diseases result from the mutation of a single gene, and others have a more complex origin, including multiple genetic and environmental factors.

1 THE HUMAN GENOME

The human genome consists of approximately 3×10^9 nucleotides and includes up to 10^5 genes. Each gene usually has a coding region that determines the sequence of amino acids in the final protein product. This coding region is split into *exons,* with intervening sequences called *introns.* A *promoter* contains DNA sequences that direct the synthesis of an RNA copy for the entire gene, and splicing signals then control the precise removal of the introns. Each end of the spliced RNA, now called mRNA, is further modified chemically before the molecule is used as a template for protein synthesis (see Figure 1).

2 DNA VARIATIONS

Except for identical twins, no two people have exactly the same sequence of DNA. Since less than 5% of the genome consists of protein coding sequence, most person-to-person DNA variations have no apparent effect. Other DNA differences do affect the phenotype, but most of these affect normal (variable) characteristics such as hair and eye color. A small but important fraction of DNA variations result in a phenotype that is not considered "normal," such as a genetic disease. In DNA analysis for diagnosis of genetic disease, it is important to distinguish between normal DNA variations ("polymorphisms") and the alteration that causes the disorder ("mutation").

2.1 MUTATIONS

Several thousand of the genes in the human genome can cause disease if disrupted in some way. A DNA mutation may be a deletion, insertion, duplication, inversion, or other rearrangement (Figure 1), and the extent of a mutation may range from a single nucleotide to a whole chromosome. If the mutation occurs in the protein coding region of a gene, the amino acid sequence may be changed, or even shortened if a premature stop codon is created (Figure 2). A mutation can also affect other portions of a gene such as the promoter or processing signals (Figure 1). The result of a mutation in a particular gene may be that an altered protein is made, that the protein is not made at all, that the wrong quantity of the protein is made, or that the protein is made at the wrong time in development.

2.2 POLYMORPHISMS

Polymorphisms, that is, DNA variations among individuals, are usually one of two types. Single-nucleotide changes, if they happen to fall within the recognition site of a restriction endonuclease, may be detected as restriction fragment length polymorphisms (RFLPs) (Figure 3A). A number of other methods are available for the detection of other single-nucleotide alterations that do not occur within restriction endonuclease recognition sites.

The other major type of DNA polymorphism is a variation in the number of tandemly repeated sequences at a particular location in the genome (Figure 3B). The repeated sequence may be 10–60 nucleotides long, in which case the tandem array is called a "minisatellite." Groups of shorter repeats (2–5 nucleotides each) are called "microsatellites."

3 GENETIC THERAPY

Genetic therapy is the supply of a functional gene to cells lacking that function, with the aim of correcting a genetic disorder or acquired disease. Genetic therapy, or "gene therapy," can be broadly divided into two categories. The first is alteration of germ cells, that is, sperm or eggs, which results in a permanent genetic change for the whole organism and subsequent generations. This "germ line gene therapy" is not currently considered an option in humans for technical and ethical reasons. The second type of genetic therapy, "somatic cell gene therapy," is analogous to an organ transplant. In this case, one or more specific tissues is targeted by direct treatment, or by removal of the tissue, addition of the therapeutic gene(s) in the laboratory, and return of the treated cells to the patient. Several clinical trials of somatic cell gene therapy have started, mostly for the treatment of cancers and blood disorders.

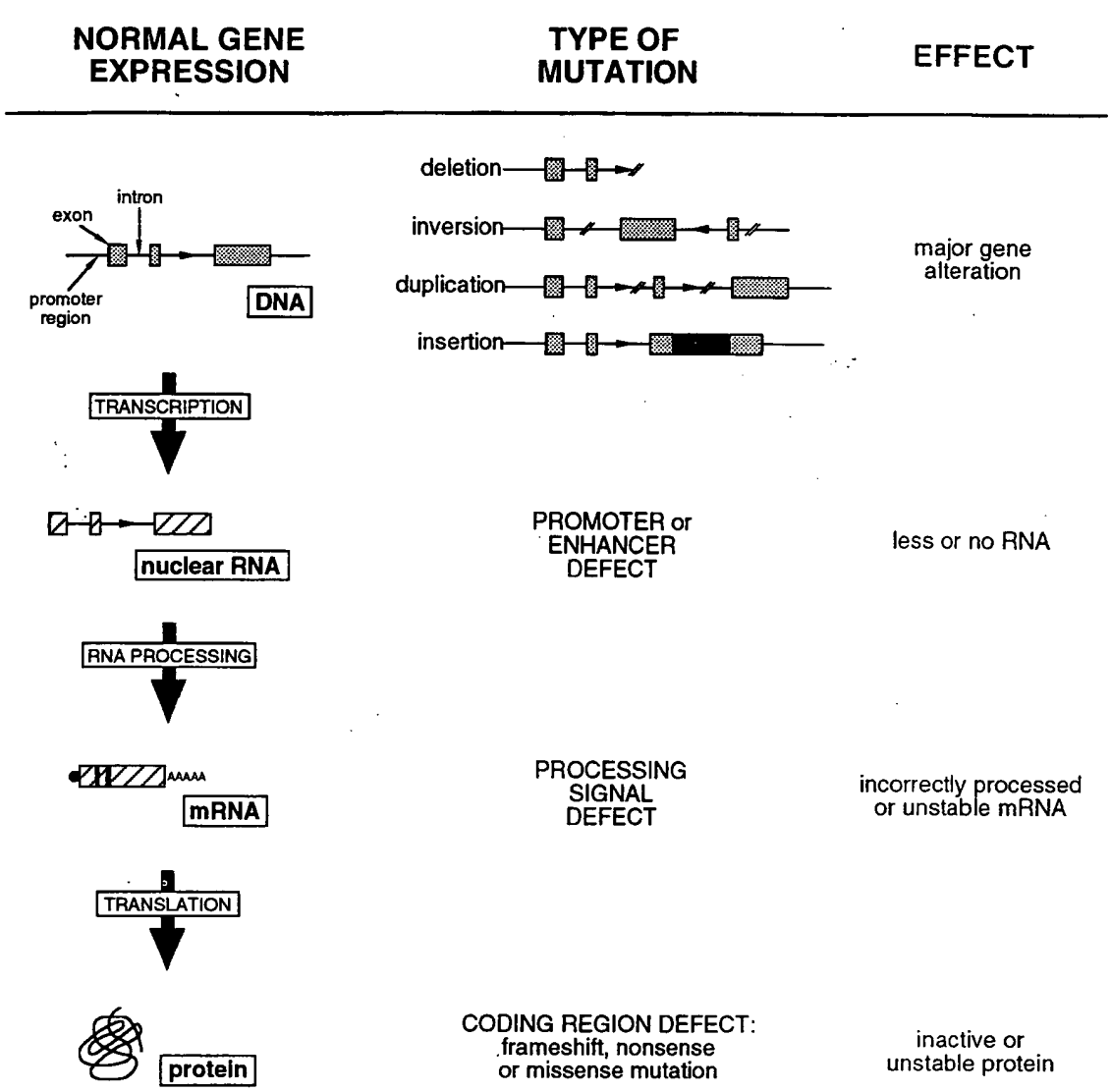


Figure 1. Some of the ways a gene can be affected by mutation. Expansion of an unstable repeat region (see Figure 4), also can affect the expression of a gene.

4 EXAMPLES OF GENETIC DISEASES

Four examples of genetic diseases are described to illustrate different aspects of human genetic disease.

4.1 Symptomless Carriers of a Childhood Disorder: Cystic Fibrosis

Cystic fibrosis has an autosomal recessive inheritance and affects approximately 1 in 2500 Caucasians. The disease is recognized at birth or in early childhood and primarily affects the lungs and pancreas. Children with cystic fibrosis are usually born to parents who do not have the disease, but each carries one copy of a cystic fibrosis mutation. In fact, many cystic fibrosis births occur without any family history of the disease.

Genetic testing methods can now detect the most common cystic fibrosis mutations, even in unaffected carriers of the disorder. It is therefore feasible to test relatives of a cystic fibrosis patient to find other carriers in the same family, or even to offer cystic fibrosis mutation screening to people who do not have a family history of this disorder.

4.2 Unstable Genetic Sequences: Fragile X Syndrome and Myotonic Dystrophy

Fragile X syndrome primarily affects males and is the most common cause of inherited mental retardation. Myotonic dystrophy is the most common adult muscle disorder, and symptoms range from being barely detectable to a severe congenital form that includes mental retardation. Both the fragile X gene and the myotonic dystrophy gene contain a polymorphic triplet repeat, that is, a group of three nucleotides repeated a variable number of times (see Section 2.2). Those with a number of repeats within the "normal" range are not affected; but in susceptible individuals, the repeat region can expand during inheritance from parent to child. Once the number of

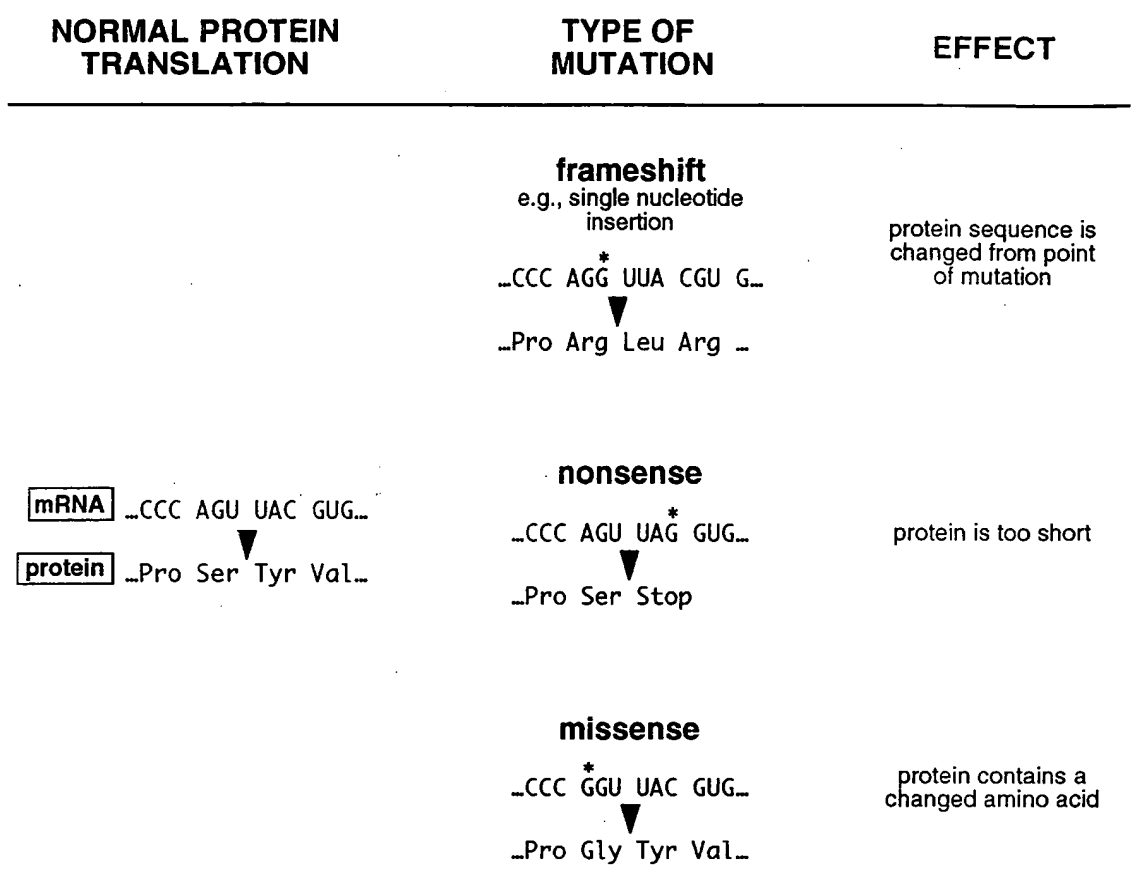
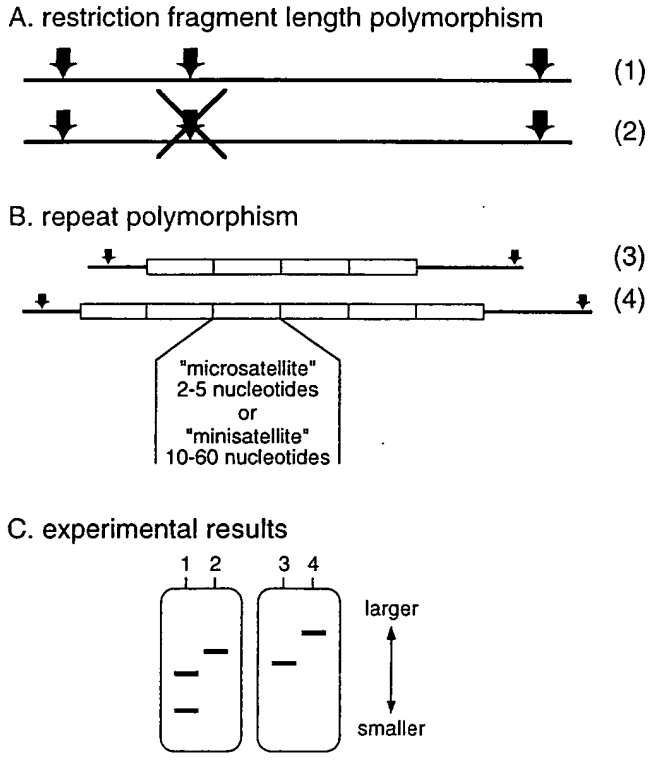


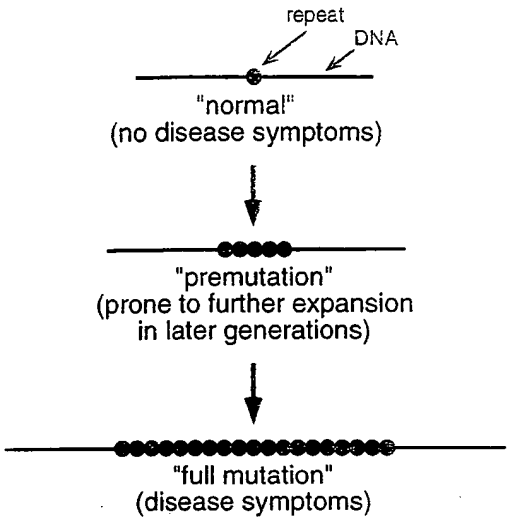
Figure 2. Some of the mutations that can affect the coding region of a gene.



triplet repeats has exceeded a certain number, the chances of having symptoms of the particular disorder, or of bearing an affected child, increase dramatically (Figure 4).

Genetic testing can reveal expanded repeat regions in fragile X syndrome or myotonic dystrophy families. Such testing can provide an estimate of the likelihood of disease in individuals or their children, although the correlation of repeat expansion and severity

Figure 3. Two major types of DNA polymorphism, and some experimental results. (A) Restriction fragment length polymorphism (RFLP). Open arrows indicate restriction endonuclease recognition sites. The presence or absence of a restriction endonuclease recognition site in a specific region of the genome determines the size of the corresponding DNA fragment after digestion by that particular enzyme. The alteration of a single nucleotide in the DNA sequence can determine the presence or absence of a restriction site. (B) Repeat polymorphism. Open boxes represent repeated sequence units that may be short (2–5 nucleotides) or longer (usually 10–60 nucleotides). Repeat polymorphisms differ across individuals in the number of repeated sequence units at a particular location. The length of the fragment containing the sequence repeat indicates the number of repeated units. (C) Experimental results. DNA fragments can be separated by size using gel electrophoresis, and visualized directly or after Southern analysis and autoradiography. The horizontal bars indicate bands that would be seen after such analysis of DNA from (A) and (B). In these examples of RFLP (left-hand panel) and repeat polymorphism (right), the lanes marked 1–4 correspond to the different DNA fragments shown in (A) and (B).



...ure 4. Expansion of unstable repeat regions resulting in disease. Some ...se genes contain a region of repeated triplet nucleotides that is polymor- ... (i.e., the number of repeats is variable), as shown in Figure 3B. ...ple with a so-called *premutation* do not have symptoms of the disorder ...selves but have an expanded repeat region that can further increase ...ze during passage from parent to child. Individuals with a "full muta- ..." are affected with disease symptoms.

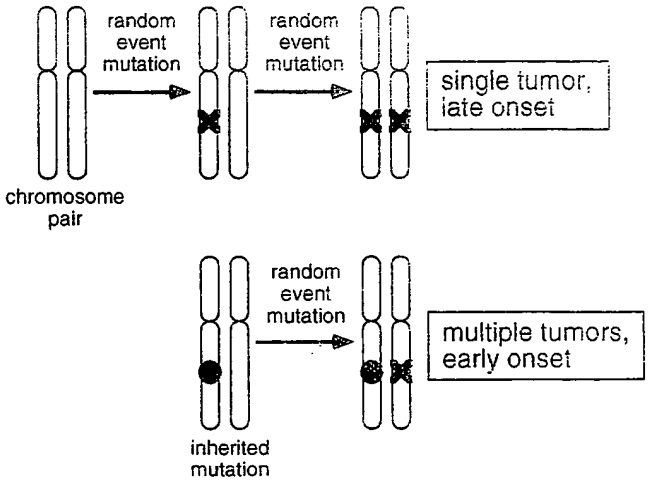


Figure 5. The "two-hit" model of cancer gene mutation. One of the events in the development of cancer may be a mutation in both copies of an oncogene or a tumor suppressor gene. It is rare for both copies of the gene to be affected by random events, and thus the outcome is usually late onset of a single tumor. If one mutation is inherited, only one further random event is required, and the individual usually experiences earlier onset of multiple tumors.

...disease is not precise. It is also possible to detect a repeat region ...t has not yet expanded enough to produce symptoms but may ...so in subsequent generations.

1.3 Presymptomatic Diagnosis of a Late-Onset Disorder: Adult Polycystic Kidney Disease

The first symptoms of adult polycystic kidney disease usually ...ppear at about age 40, and progression of the disease results in ...hronic renal failure. Renal cysts can be detected by ultrasound ...fore the onset of symptoms (approximately 70% of cases can ... detected by age 20), and genetic testing in a family affected by ...e disease can determine the presence of the adult polycystic ...dney disease gene as early as the prenatal period. This is one of ...any examples of inherited diseases that can be diagnosed by ...enetic testing before the appearance of symptoms. Most such ...sorders, however, currently have no treatment or cure.

1.4 Predisposition to Genetic Disease: Colorectal Cancer

Colorectal cancer is not the result of disruption of a single gene, ...t there is strong evidence that a series of genetic events can ...entually lead to the disease. A number of genes have been discov- ...ed that are frequently altered in colon cancer. Some of these ...enes are "oncogenes," which can become *activated* by mutation, ...nd some are "tumor suppressor genes," which normally repress ...ell growth but are *inactivated* by mutation. It is thought that ...n accumulation of multiple mutations in oncogenes and tumor ...uppressor genes can eventually lead to colorectal cancer. These ...utations may occur spontaneously or they may be inherited (Fig- ...re 5). It is not surprising, therefore, that inheritance of one of these mutations increases the risk that an individual will develop cancer. Genetic testing, particularly of someone with a family history of colorectal cancer, may reveal a mutation that is not in itself a *prediction* that cancer will occur, but rather an indication of *predisposition* to that disease.

See also Colon Cancer; DNA in Neoplastic Disease Diagnosis; Fragile X Linked Mental Retardation; Genetic Testing; Molecular Genetic Medicine; Neuropsychiatric Diseases; Oncogenes; Population-Specific Genetic Markers and Disease; Tumor Suppressor Genes.

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Human Genome: *see* Body Expression Map of the Human Genome.

These spots are derived from mouse ribosomal DNA, which is the typical repetitive sequence in a mammalian genome. The haploid and diploid genomic DNAs can be distinguished in RLGS.

5. When methylation-sensitive enzyme is used, the methylated state of genomic DNA can be screened.
6. When CpG-rich enzymes (*Not* I, *Bss*HII, etc.) are used, CpG islands near the genes are preferentially screened. For example, 89% of all *Not* I spots are located on or near transcripts. Thus, RLGS is very advantageous compared with hybridization-based and PCR-based genome scanning methods.

APPLICATIONS

The RLGS method can be used in two ways. One is for genome scanning to determine the copy number of each locus corresponding to a spot, using methylation-insensitive enzyme as a restriction landmark. The other use is for the detection of DNA methylation throughout the genome, using methylation-sensitive enzyme.

Generally, the only known site for DNA methylation is the 5-position of the cytosine residue (^{5Me}C), which is preferably located in the palindromic dinucleotide 5′CG3′/5′GC3′. In ^{5Me}CpG methylation-insensitive enzymes such as *Pac* I, *Swa* I, and *Sse*8387I, the cleavage is not affected by DNA methylation in the vertebrate genome. Thus when these enzymes are used on the RLGS profile, the intensity of the spots depends completely on the copy number of the corresponding locus. On the other hand, when ^{5Me}CpG-sensitive enzyme is used as restriction enzyme A, the intensity of the RLGS spots changes depending on DNA methylation, as shown in Figure 3a.

For example, high speed genetic mapping that allows the identification of the chromosomal location of many RLGS spots has been published. Taking advantage of one feature of RLGS—namely, that spot intensity reflects the copy number of the landmark—amplification in cancer DNA and loss of heterozygosity can be detected. Figure 3b shows an example of systemic detection of DNA methylation changes at the *Not* I site during aging. All three patterns were produced from the liver DNA of the C57BL/6 inbred mouse strain at three stages: birth, 8 weeks, and 45 weeks. The spot represented by the arrowhead is altered in intensity, indicating that DNA methylation state on the restriction landmark *Not* I is variable. The RLGS method described here can be applied to many biological fields, such as cancer research, high speed genome mapping (large number of analyzable loci), identification of individuals, and phylogenetic analysis of species. Thus, the RLGS method is very useful for scanning not only the change of copy number of each locus, but also the change in DNA methylation.

See also AUTOMATION IN GENOME RESEARCH; DNA SEQUENCING IN ULTRATHIN GELS, HIGH SPEED; RESTRICTION ENDONUCLEASES AND DNA MODIFICATION METHYLTRANSFERASES FOR THE MANIPULATION OF DNA.

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RETINOBLASTOMA, MOLECULAR GENETICS OF

Brenda L. Gallie and John Wu

Key Words

Loss of Heterozygosity Loss of large regions of one chromosome, detected by loss of polymorphic markers on that chromosome: that is, the cells appear to have become homozygous for all markers within the region.

Retinoma Benign retinal tumors resembling treated retinoblastoma that do not continue to grow, but spontaneously cease proliferation; caused by mutations in *RB1*.

Transcription Factor Proteins that regulate the expression of genes by binding in various protein complexes and DNA.

Viral Oncoproteins Proteins of the DNA tumor viruses that induce cellular proliferation and tumors in animals by binding to normal host cellular regulatory proteins, such as the retinoblastoma protein (pRB).

Retinoblastoma, a rare tumor of the infant retina, has become important for understanding cancer in general. Studies of retinoblastoma tumors revealed a previously unsuspected mechanism of cancer initiation, loss of heterozygosity. The retinoblastoma gene (*RB1*) was the first tumor suppressor gene to be defined, and it has turned out to be a transcription factor involved in the regulation of how almost all adult mammalian cells divide. It is not yet known why mutations of *RB1* initiate specific tumors such as retinoblastoma but do not cause a general tumor predisposition.

1 THE DISEASE

Retinoblastoma is a rare pediatric tumor that arises from the immature neuroectodermal cells of the developing retina, with an estimated incidence of one in 20,000 births. About half the patients have heritable retinoblastoma and can transmit this tendency as an autosomal dominant trait; these children usually have bilateral and multifocal tumors, diagnosed on average at about one year of age. Some children with a heritable mutation may develop only one

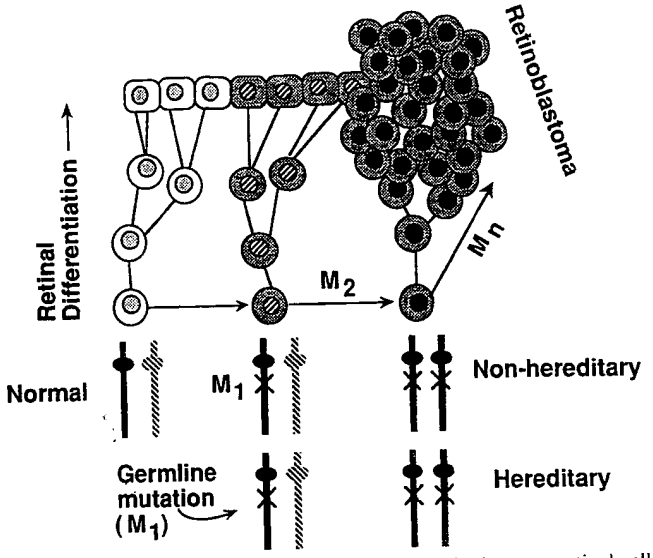

Figure 1. Two mutations (M_1 and M_2) occurring in the same retinal cell are needed to initiate the process of malignant transformation. For malignant progression, however, additional mutations (M_n) must accumulate. In nonhereditary retinoblastoma, both M_1 and M_2 occur in the same somatic retinal cell. In hereditary retinoblastoma, M_1 has already occurred in the germ line; the additional mutation (M_2) in retinal cells initiates the malignant process.

tumor in one eye, and some mutation carriers develop no tumors at all. All nonhereditary cases develop only one unilateral, solitary retinoblastoma tumor. These manifestations of the disease also can be diagnosed from birth to early childhood, although on average they occur at an older age than the heritable tumors.

Statistical analysis of the different mean ages of diagnosis of hereditary and nonhereditary retinoblastoma led Knudson to hypothesize that the rate-limiting number of mutations for the initiation of this tumor is at least 2. In hereditary retinoblastoma, the first mutation is present in every cell of the patient, and only one somatic mutation is required to initiate the cancer; in nonhereditary tumors, the two mutations must occur in the same somatic retinal cell that eventually is transformed to malignant neoplasm. Comings extended this idea to suggest that the mutations could involve the two allelic homologues at the same locus, illustrated in Figure 1. These hypotheses were later supported by the demonstration of frequent loss of an allele from tumors and mutations in the retained allele(s) at the retinoblastoma locus (*RB1*).

The mechanism of loss of an allele was first recognized to initiate cancer in retinoblastoma. Subsequent studies searched for chromosomal regions in which a similar loss of heterozygosity (LOH) had occurred in tumors of specific tissues, and thus other tumor suppressor genes were identified, such as the Wilms' tumor gene and genes predisposing to adenomatosis of colon and rectum. Several chromosomal regions that show LOH in tumors are suspected of containing tumor suppressor genes not yet discovered. The tumor suppressor gene, p53, also shows LOH in many tumor types.

In heritable retinoblastoma, the first mutation is found heterozygously in germ cells of the individual. The mutation may be inherited, but more commonly it has newly arisen on the father's allele. It is not known why new mutations are more common in fathers' chromosomes than in mothers'. Ninety percent of children with a heterozygous *RB1* constitutional mutation will develop retinoblastoma, a relative risk (RR) of 40,000 compared to the general population. This accounts for the autosomal dominant pattern of t sion of the predisposition to retinoblastoma, although th that arise have mutated both alleles. The same individual RR of 500 to later develop other solid tumors, most osteogenic sarcomas. Radiation, the conventional treatm treating retinoblastoma in eyes with salvageable vision this risk a further tenfold.

Since only a few patients have a family history of retinobl most children are diagnosed when one eye contains large tu causing the white pupil or "cat's eye" that is the classic p sign. When such eyes are removed, cure is achieved if cells have extended outside the eye. Smaller tumors presen eye with useful vision can often be cured by combinat chemotherapy, laser therapy, cryotherapy, and focal radiat

Rare retinal tumors resembling retinoblastomas but not beh in a malignant fashion arise because of *RB1* mutations called retinomas. It is hypothesized that these growths initiated by the loss of *RB1* at a late stage of retinal differen when insufficient cell divisions remain before terminal diffe tion to permit accumulation of the extra mutations in other genes required for full malignancy. At the other extreme, multi retinoblastoma tumors that have spread outside the eye have a poor prognosis, demonstrating mutations in multiple additi growth-related genes. These include, rarely, amplification of N-*myc* proto-oncogene and, commonly, overexpression of the tidrug resistance gene, p170, which has contributed to the success of chemotherapy in retinoblastoma in the past.

Since infant relatives of retinoblastoma patients are also at to develop retinoblastoma tumors, they are repeatedly exami often under anesthetic, to ensure the early discovery of tum Identification of the *RB1* mutation(s) in the tumor and constitutio cells of the first affected child makes possible simple molec screening of relatives to determine which one has the famil mutation. Those unaffected will not require the intense surveillan New technology to identify each family's mutation based on aut mated DNA sequencing will soon be available, to permit this to as a health service.

2 THE GENE AND PROTEIN

The *RB1* gene spans 180 kilobases of chromosome 13 band q1 and consists of 27 exons. A 4.7 kb *RB1* transcript is expressed all adult tissues. The *RB1* gene codes for a nuclear phosphopro of 928 amino acids (pRB).

There is evidence that *RB1* expression is regulated at a transcri tional level by autoregulatory feedback. First, mutant *RB1* mR is undetectable in constitutional cells of individuals heterozygo for germ line *RB1* mutations, in which the normal allele contribut a normal pRB, but mRNA with the same mutation is easily dete able in retinoblastoma cells with no functional protein. Secon transient expression of pRB suppresses expression from an *RB1* promoter fused to a reporter construct. The *RB1* promoter region TATA-less and contains consensus motifs for transcription factor ATF, Sp1, and E2F.

The apparent molecular mass of pRB (110–116 kDa) depend on the state of phosphorylation of serine and threonine residu which is tightly regulated in the cell cycle. The protein is hypoph phorylated in G_0 and early G_1, but hyperphosphorylated in late G S, G_2, and M phases. The hypophosphorylated form binds th viral transforming proteins, adenovirus E1A, SV40 large T, and papillomavirus E7. The transforming potential of these viral onc

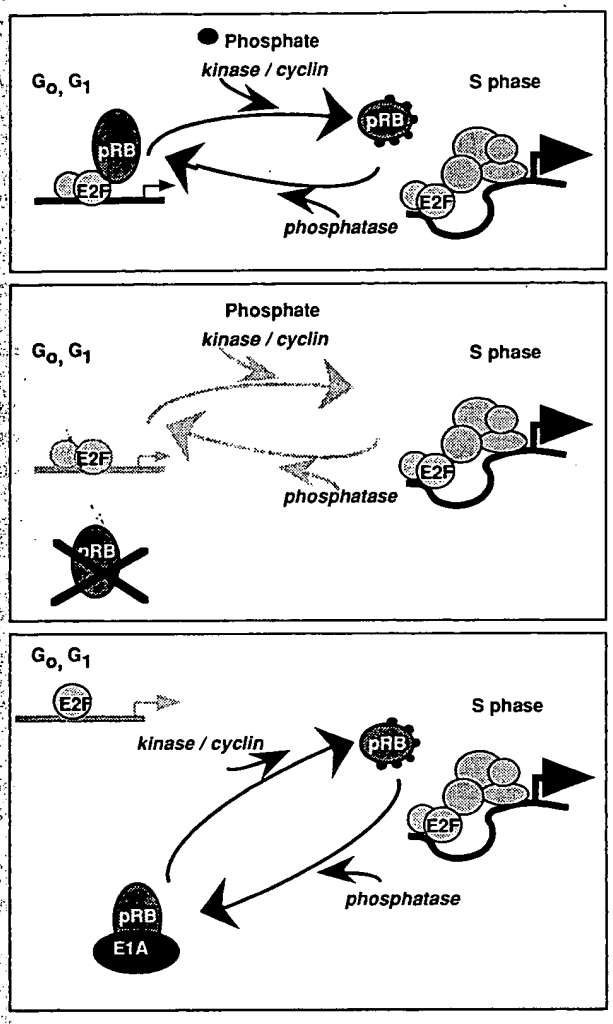

Figure 2. (A) The active, hypophosphorylated retinoblastoma protein (pRB) binds and inhibits E2F complexes during G_0 and G_1. pRB is phosphorylated by cell-cycle-specific kinases, complexed to cyclins, as the cell enters S phase, releasing E2F to activate transcription of responsive genes. After mitosis, pRB is dephosphorylated, making it again available for binding to E2F during G_1 phase. (B) Mutations affecting pRB disable it from binding E2F, rendering the cell incapable of achieving the terminal differentiation normal for retina. (C) Viral oncoproteins bind and sequester pRB to achieve malignant transformation.

proteins, which lies in part in their ability to bind and sequester pRB away from its normal role of negative growth regulation, is dependent on critical protein domains. This suggests that hypophosphorylated pRB is the active form, which performs its function in G_0 and G_1 phases of the cell cycle, as outlined in Figure 2. The binding of pRB to the viral oncoproteins depends on the integrity of two separate domains, which together form a "pocket" for binding. A number of other cellular proteins also bind to the viral oncoproteins in the course of cellular transformation, including the p107 protein, homologous to pRB, and the tumor suppressor gene p53. Thus there is more than one pathway controlling cellular proliferation, and the sum of them all must be neutralized to achieve malignant transformation.

During G_0 and G_1, hypophosphorylated pRB exerts its negative effect on cellular growth by binding to transcription factors, rendering them unavailable for modification of transcription. The cell is thus blocked from proliferation and not provided an opportunity to differentiate. In late G_1, pRB is phosphorylated by cell-cycle-specific kinases related to $p34^{cdc2}$ and $p33^{cdk2}$, becomes inactive, and no longer binds to positive growth regulators, enabling these factors to promote cell proliferation.

Some of the growth response genes regulated by pRB are known. The expression of c-*fos*, itself a transcription factor responsible for early response to growth signals, is repressed by pRB, acting through the "retinoblastoma control element" (RCE) promoter consensus sequence. Hypophosphorylated pRB binds to complexes containing the transcription factor E2F, and as the cell progresses through late G_1, hyperphosphorylation of pRB releases E2F to transactivate growth response genes, including c-*myc*.

Introduction of *RB1* cDNA into tumors negative for pRB, including retinoblastoma tumors, shows variable and only partial reversal of the malignant phenotype. Although retinoblastoma initiation requires the loss of both alleles in *RB1*, additional mutations are likely to be involved in the malignant transformation process, probably maintaining malignant growth despite reconstitution with a wild-type *RB1*.

The important role of pRB in development and differentiation has been demonstrated in transgenic mice homozygous for *RB1* mutant alleles. These mice die in utero, manifesting abnormalities in the hematopoietic and nervous systems related to the failure of terminal differentiation. This set of symptoms might reflect a distinct function of *RB1* in promoting differentiation, in contrast to a role of negative growth regulation. Surprisingly, the heterozygous mice do not develop retinoblastoma, but instead develop pituitary malignancy.

Mutations leading to tumors occur throughout *RB1*, with no "hot spots"; most result in truncated proteins that are not detectable in retinoblastoma tumors. A significant proportion of other types of tumor, such as breast cancer, small-cell carcinoma of lung, and bladder cancer, also have mutations in the *RB1* gene. The *RB1* mutations in these tumors likely contribute to tumor progression by providing a selective growth advantage after the tumor is initiated. Individuals with germ line *RB1* mutations do not appear to have increased incidence of all the tumors types that show *RB1* mutations, although they do have increased incidence of specific tumor types, such as osteosarcoma.

3 UNANSWERED QUESTIONS

The exact role of *RB1* in the regulation of cellular proliferation and differentiation awaits full elucidation. Since *RB1* is expressed in most normal adult cells, interacts with general transcriptional factors, and plays a role in cell cycle progression at the G_1/S transition, it is hard to explain why only a small number of tissues are susceptible to malignant transformation in individuals with a germ line *RB1* mutation. One suggested explanation is that pRB interacts with specific factors in different tissues at critical developmental stages, with the result that the effect of the loss of *RB1* depends on the type of tissue and developmental stage. There may be redundant pathways available for growth control. The presence of pRB is not essential for progression of the cell cycle, since tumor cells that are $RB1^-$ traverse the cell cycle without hindrance. Another explanation might be that *RB1* is essential for the survival of cells of most tissues, in which loss of *RB1* is lethal. Only the few tissues that are susceptible to malignant change have redundant pathways for survival when the loss of *RB1* causes dysregulated cell proliferation.

The results observed in *RB1*⁻ transgenic mice have raised more questions. The important role of *RB1* in development is established, but the tissues affected are unexpected, since there is no increase in hematopoietic or neurological malignancies in individuals with germ line *RB1* mutation. The question is whether these tissues are uniquely sensitive to the loss of *RB1* or whether, perhaps, they are affected just because they are the first to enter terminal differentiation; in the latter case, other developing tissues would also be affected later, if the mice survived long enough. The failure to detect retinoblastoma in heterozygous *RB1*⁻ mice may reflect the requirement for the second allele to be mutated in a developmental window that may be too short in the species studied. This possibility, however, would not account for the occurrence of pituitary tumors.

See also CANCER; HUMAN GENETIC PREDISPOSITION TO DISEASE; ONCOGENES; TUMOR SUPPRESSOR GENES.

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RETINOIDS

Robert R. Rando

Key Words

Retinoid A diterpene polyene derivative related to and including vitamin A.

Visual Cycle The sequence of biochemical reactions beginning with the photochemcal cis-to-trans isomerization of the 11-*cis*-retinal Schiff base chromophore of rhodopsin and terminating with the enzymatic resyntheses of 11-*cis*-retinal.

Visual Transduction The sequence of biochemical events, initiated by the absorption of light by rhodopsin, which lead to the hyperpolarization of photoreceptors and the initiation of the visual response.

The retinoids are very important in cellular physiology. Their most completely understood roles are found in visual signal transduction in animals and in energy production in halophilic bacterial. The recent discovery of the cell growth, maintenance, and developmental roles of the retinoic acids adds a new and exciting chapter to the biology of the retinoids. All the retinoids are diterpene polyene derivatives, and as such their activities are often controlled by reversible double-bond isomerization reactions involving mono-*cis* and all-*trans* isomers. This entry explores the centrality to vision of double-bond isomerization reactions of retinoids. The possible relationship of insights drawn from this work to the hormonal roles of the retinoic acids is also described.

1 PHOTOISOMERIZATION OF RHODOPSIN AND VISUAL TRANSDUCTION

Vision is initiated when the visual pigment rhodopsin absorbs a photon of light, leading to the cis-to-trans isomerization of the protonated 11-*cis*-retinal Schiff base chromophore (Figure 1). 11-*cis*-Retinal, a diterpene retinoid related to vitamin A (all-*trans*-retinol), is covalently linked to the apoprotein opsin at the active site lysine residue. The photochemical isomerization of the chromophore in rhodopsin leads to a series of spectrally defined intermediates, resulting in the eventual hydrolysis of all-*trans*-retinyl Schiff base to produce all-*trans*-retinal and opsin as shown in Figure 1. The photoisomerization reaction is reported to be essentially complete in only 200 fs. Incoherent noise analysis has placed an upper limit on the rate of thermal isomerization of rhodopsin in the neighborhood of cattle, producing in the rods a signal-to-noise ratio of approximately 1. One of the spectroscopically defined intermediates, metarhodopsin 2, is the intermediate (R*) that transmits the information that light has been absorbed by interacting with the next molecule in the visual cascade, the retinal G protein, transducin.

The interaction of R* with transducin results in catalysis of the exchange of GDP for GTP at the active site of transducin. The transducin–GTP complex activates a rod outer segment phosphodiesterase specific for c-GMP which can hydrolyze c-GMP at the diffusion-controlled limit. This hydrolysis is thought to lower the free concentration of this effector. Since the sodium ion permeability of the rod outer segment sodium channels is gated by c-GMP, hydrolysis of c-GMP results in the hyperpolarization of the rod outer segments. This membrane potential change is transmitted to other nerve cells in the retina and communicated to the brain, where the visual image can then be constructed.

The structure of metarhodopsin 2 is of substantial interest because this molecule propagates the light signal. Moreover, rhodopsin represents the best understood member of the large family of seven-transmembrane helical receptors, and further molecular insights into its mode of action are likely to be of general significance. Of all the spectroscopically defined bleaching intermediates of rhodopsin, only metarhodopsin 2 possesses a deprotonated Schiff base. Experiments in which a single methyl group is incorporated into the active site lysine residue show that Schiff base deprotonation is obligate in the form of activated rhodopsin capable of fruitfully interacting with transducin. Active site mutated rhodopsin undergoes facile photoisomerization and yields the early photointermediates up to metarhodopsin 1. The long-lived metarhodopsin 1 does not proceed to metarhodopsin 2, cannot activate transducin, nor can it be phosphorylated by rhodopsin kinase. The idea that Schiff base deprotonation of rhodopsin is a key transduction event has gained considerable support from site-specific mutagenic studies. Recent studies in which the active site lysine is removed altogether bring the matter to a satisfying conclusion. Mutant opsins in which the active site lysine residue has been substituted for a glycine or alanine residue are constitutively active with respect to the catalysis of GTP–GDP exchange in transducin. Since these mutants do not have a positive charge at their active sites; they are able to form metarhodopsin 2 in the absence of chromophore and photoisomerization. This suggests that the role of 11-*cis*-retinal is to prevent protonated Schiff base formation and the turning off of a possible

MICROBIOLOGY

An Introduction

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About the cover: A technician is isolating plasmids, which are tiny circles of DNA found in bacteria. The plasmids are dissolved in a dye solution that fluoresces pink under ultraviolet light. Genetic engineering using plasmids is revolutionizing the biological sciences and industry (see pages 226–229 and 704–707).

Figure acknowledgments begin on page 749.

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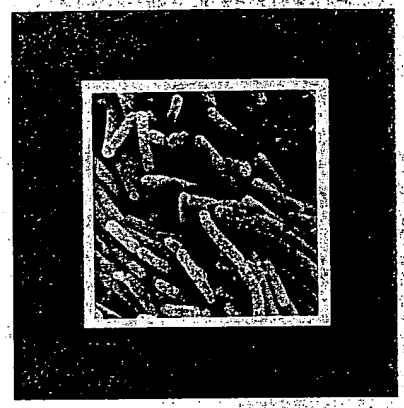


8

Microbial Genetics

Objectives

After completing this chapter you should be able to

- Define the following: genetics; chromosome; gene; genetic code; mutagen; genetic recombination.
- Describe DNA replication.
- Describe protein synthesis.
- Classify mutations by type.
- Describe the actions of mutagens on DNA.
- Outline how mutations are identified by direct and indirect selection.
- Compare mechanisms of genetic transfer in bacteria.
- Define plasmids and discuss their functions.
- Outline how recombinant DNA is produced in vitro and list its potential uses.
- Explain the regulation of gene expression through feedback inhibition, induction, and repression.

All the characteristics of microorganisms, including their morphology, metabolism, behavior, and pathogenicity, are inherited characteristics. Individual organisms transmit these characteristics to their offspring through genes, which are units of heredity that contain the information for determining these characteristics. **Genetics,** the science of heredity, is the study of what genes are, how they carry information, how they are replicated and passed to further generations of cells or passed between organisms, and how their information is expressed within an organism to determine the particular characteristics of that organism.

STRUCTURE AND FUNCTION OF THE GENETIC MATERIAL

Genes consist of DNA, deoxyribonucleic acid. In Chapter 2, we saw that DNA is a macromolecule composed of repeating units called *nucleotides*. You may recall that each nucleotide consists of a nitrogenous base (adenine, thymine, cytosine, or guanine), deoxyribose (a pentose sugar), and a phosphate group. How the nucleotides are arranged in a DNA molecule may be reviewed in Figure 2–19.

The DNA within a cell exists as two long strands of nucleotides twisted together to form a double helix. Each strand is a string of alternating sugar and phosphate groups, and to each sugar is attached a nitrogenous base. The two strands are held together by hydrogen

bonds between their nitrogenous bases. The bases are paired in a specific, complementary way. Adenine (A) pairs with thymine (T), and cytosine (C) pairs with guanine (G). The thing to remember here is the notion of hydrogen bonding between the nitrogenous bases of the two strands leading to the formation of specific base pairs, A–T and C–G.

Later in this chapter, we will describe several important experiments that clearly identified DNA as the genetic material of cells. By saying that DNA is the genetic material, we mean that this molecule carries the cell's history as well as its future. The information locked within DNA sequences determines the characteristics of the cell and transfers these characteristics to subsequent generations of cells. But DNA does not work alone. A gene is not "expressed" (the chemical it codes for, usually a protein, is not actively produced) until RNA reads the code and directs the proper assembly of amino acids to form the protein prescribed by the gene. Proteins are so important that much of cellular machinery is concerned with translating the DNA of genes into specific proteins. The genetic information in a region of DNA is transcribed (copied) to produce a specific molecule of RNA. The information encoded in RNA is then translated into a specific sequence of amino acids that form a newly produced protein.

A **gene** may be defined as a segment of DNA (or a sequence of nucleotides in DNA) that codes for a functional product. The final product can be a molecule of ribosomal RNA, for example. Usually, however, the final product is a protein. Thus, DNA is transcribed to produce a specific molecule of RNA, and the information encoded in RNA is translated into protein. This can be symbolized as follows:

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{Translation}} \text{Protein}$$

This flow of genetic information within a cell and between generations of cells is summarized in Figure 8–1.

DNA is a particularly suitable molecule to act as the cell's genetic library. Looking at the outside of the molecule, we tend to focus on the repeating alteration of sugars and phosphates. But inside, purine and pyrimidine bases are arranged in specific sequences that form strings of information—the genes that make up the books in this library. These books use a genetic alphabet with only four letters: the bases A, T, G, and C. But a gene with 1000 bases can be arranged in 4^{1000} different ways. This is how the genes can carry so much information; they code it within the multitude of base sequences available to the DNA molecule.

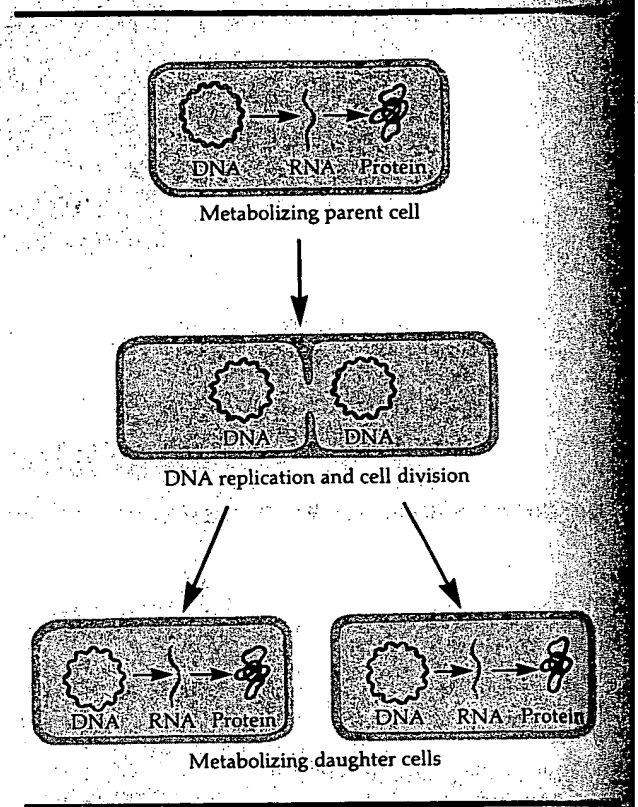


Figure 8–1 Flow of genetic information within a cell and between generations of cells.

Besides being able to store information, DNA can also carry this information from cell to cell and from generation to generation, because the specific sequence of nucleotides contained in the DNA usually duplicates accurately each time the cell divides. We shall see in the next section how this amazing feat is made possible by the distinctive structure of DNA, with its double helix formed by two complementary nucleotide chains.

Because the two DNA strands can open up and rejoin easily, genes are accessible to the rest of the cell. But the genes are not removed from the DNA

make proteins. Instead, copies of the genes are scribed into RNA messengers, which carry instructions to the ribosomes to make proas needed.

Finally, DNA can change, or mutate. Although mutations can kill the cell, others permit the spring a better chance of survival should they be jected to new environments or stressful conditions. Thus, DNA's ability to mutate is in the long an advantage that contributes to the evolution the organism.

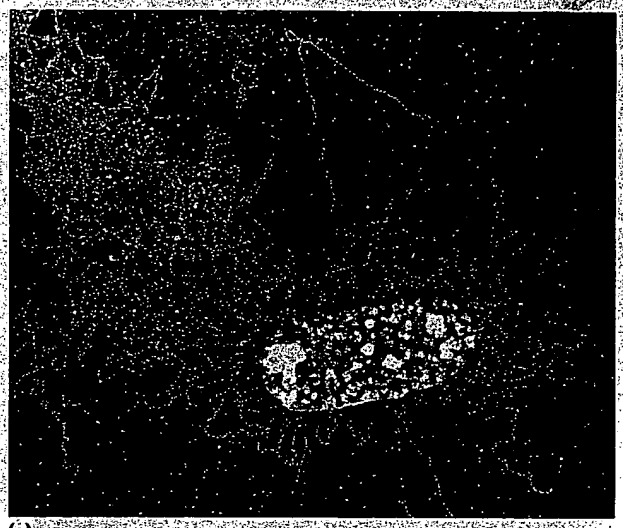
Before we discuss the details of how DNA repfunctions, and changes, let us briefly dis- the way the DNA is arranged within the —the structure of the chromosomes.

NA AND CHROMOSOMES

though the structure of DNA is fairly well underod, the packaging of DNA in chromosomes is . Evidence available so far suggests that the NA in each chromosome—even in the large, comchromosomes of eucaryotes—exists as one g double helix associated with various kinds of otein that regulate genetic activity.

The DNA of *Escherichia coli*, the common bacum of the human large intestine, is contained a typical procaryotic chromosome: a single long olecule of DNA with no nuclear membrane to lose it (Figure 8–2a). The chromosome is attached one or several points to the plasma membrane. *coli* DNA has about 4 million base pairs and is out 1 mm long—1000 times longer than the entire ll. However, DNA is also very thin and tightly cked inside the cell as a twisted, coiled macrolecule that takes up about 10% of the cell's volne. Bacterial DNA is circular—a closed loop with free ends. Some proteins (including RNA polyerase and others) are associated with bacterial NA. Because it is a very long molecule, it can ntain large amounts of information. Essentially, contains the blueprint for the entire cell.

Eucaryotic chromosomes (Figure 8–2b) contain NA that is even more highly coiled (condensed) an procaryotic DNA, and they contain much ore protein than procaryotic chromosomes do. caryotic cells have histone proteins that are anged in a very regular way with their DNA.



(a)

(b)

Figure 8–2 **(a)** Electron micrograph of a procaryotic chromosome. The tangled mass and looping strands of DNA emerging from this disrupted cell of *Escherichia coli* are part of its single chromosome (×6000). **(b)** Electron micrograph of a procaryotic chromosome. This human chromosome is but one of 46 chromosomes found in a normal human cell. Note the individual strands of protein–DNA fibers (×28,000).

The detailed structure of the eucaryotic chromosome, and the precise arrangement of DNA with proteins, is still under investigation. Researchers feel that an understanding of the physical and chemical arrangement of the protein-wrapped DNA is likely to reveal how genes are turned on and off to produce crucial proteins when needed.

We will now examine how DNA functions as the genetic material through replication and protein synthesis.

DNA REPLICATION

The complementarity of the nitrogenous base sequences in the two strands of the double helix provides the key to understanding DNA replication. In the process of replication, the two strands of the original DNA double helix separate from each other (Figure 8–3). The separation point where new strands will be synthesized is called the *replication fork*. Following separation, new nucleotides are matched up to the exposed portions of the original strands according to the complementary rules of base pairing. If thymine is present on the original strand, only adenine can fit into place on the new strand; if guanine is present on the original strand, only cytosine can fit into place on the new strand; and so on. The new nucleotides are joined to each other by enzymes called *DNA polymerases*. Initially, only short fragments of DNA are synthesized. An enzyme called *DNA ligase* joins the fragments together, producing two complete strands of DNA.

The net result is that two new strands of DNA are synthesized, each having a base sequence complementary to one of the original strands of DNA. Since each new double-stranded DNA molecule contains one original strand and one new strand, the process of replication is referred to as **semiconservative replication**. Along the length of the DNA molecules, there are specific sites where replication is initiated; in bacterial DNA, there is usually one initiation site. In some cases, replication moves in one direction; other times it moves in two. Bacteria have circular molecules of DNA and in some bacteria, such as *E. coli*, replication is bidirectional. Two replication forks move in opposite directions around the DNA molecule and eventually meet to complete replication (Figure 8–4).

RNA AND PROTEIN SYNTHESIS

Although the previous section explains how DNA stores genetic information in nucleotide sequences and replicates itself by a process involving base pairing, it does not explain how the information in DNA is translated into the proteins that control cell activities. In a process called transcription, much of the genetic information in DNA is copied, or encoded, by a complementary base sequence of RNA. The RNA message is then used by the cell to synthesize specific proteins through the process of translation. We will now take a closer look at these two processes.

Transcription

In the process of **transcription,** a strand of RNA called **messenger RNA (mRNA)** is synthesized by using a specific portion of the cell's DNA as a template (Figure 8–5). In other words, the genetic information stored in the sequence of nitrogenous bases of DNA is rewritten so that the same information appears in the nitrogenous base sequence of RNA. As in DNA replication, a G in the DNA template dictates a C in the mRNA being made; a C in the DNA template dictates a G in the mRNA; and a T in the DNA template dictates an A in the mRNA. However, an A in the DNA template dictates a uracil (U) in the mRNA, since RNA does not contain T; it contains U instead. If, for example, the template portion of DNA has the base sequence ATGCAT, then the newly synthesized mRNA strand would have the complementary base sequence UACGUA. This may be represented as follows:

Template DNA		mRNA
A		U
T		A
G		C
C	Transcription →	G
A		U
T		A

The process of transcription requires an enzyme called *RNA polymerase* and a supply of RNA nucleotides. Only one of the two DNA strands serves as the template for RNA synthesis. This is called the *sense strand*. The other strand, the *antisense strand*,

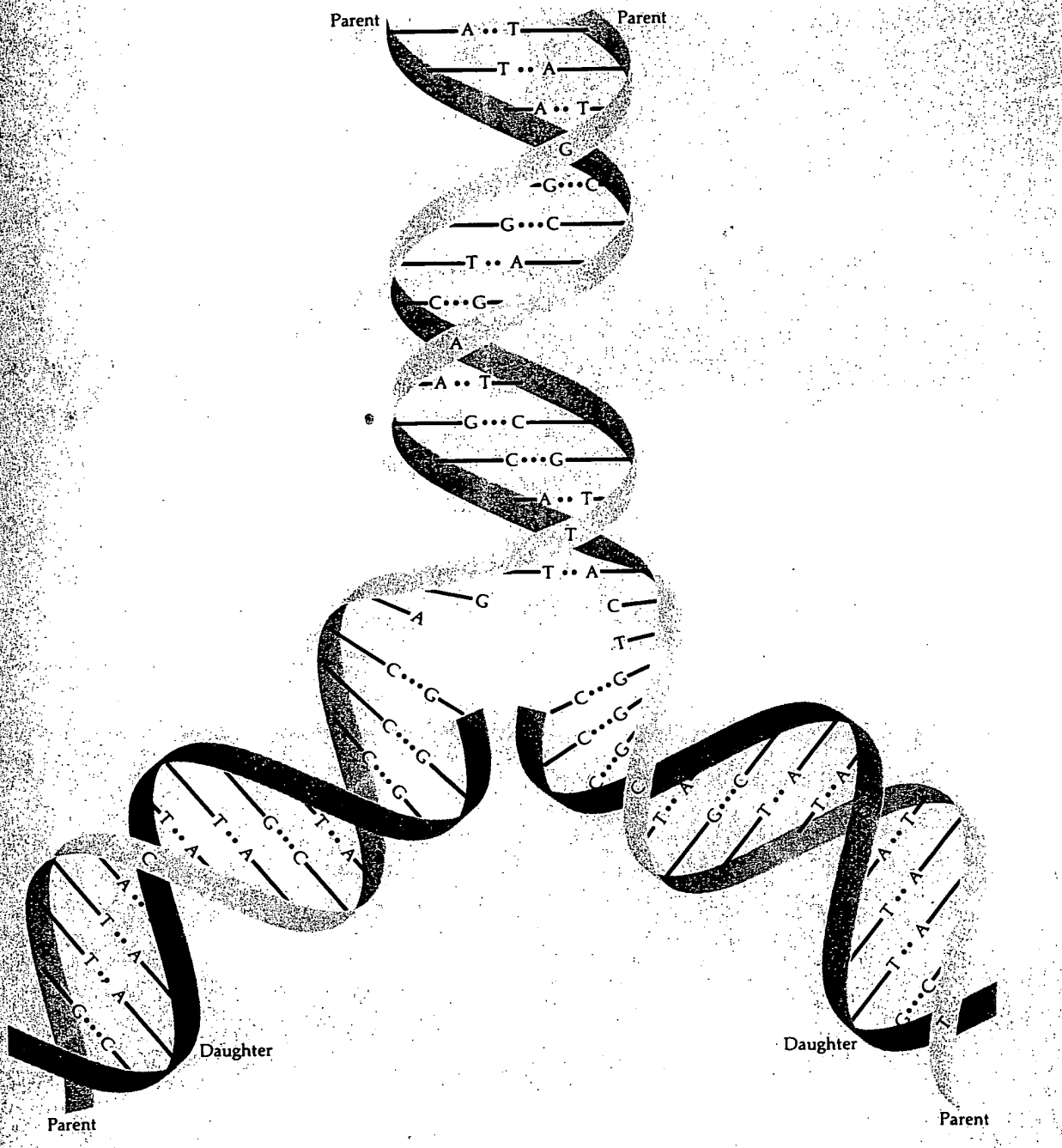
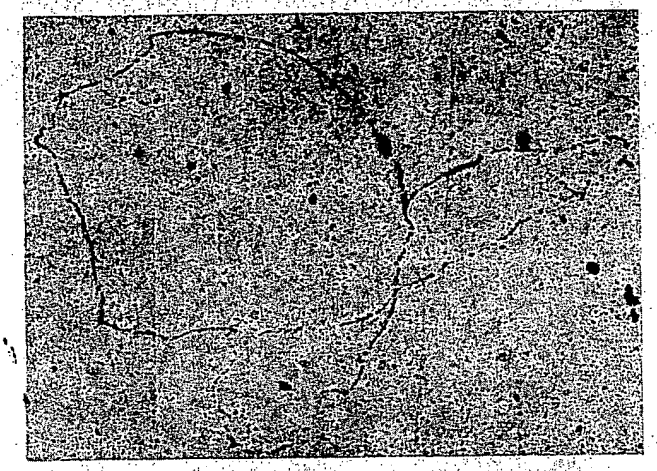
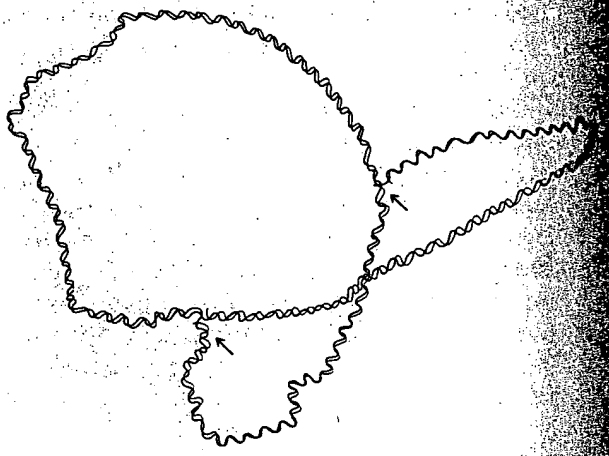
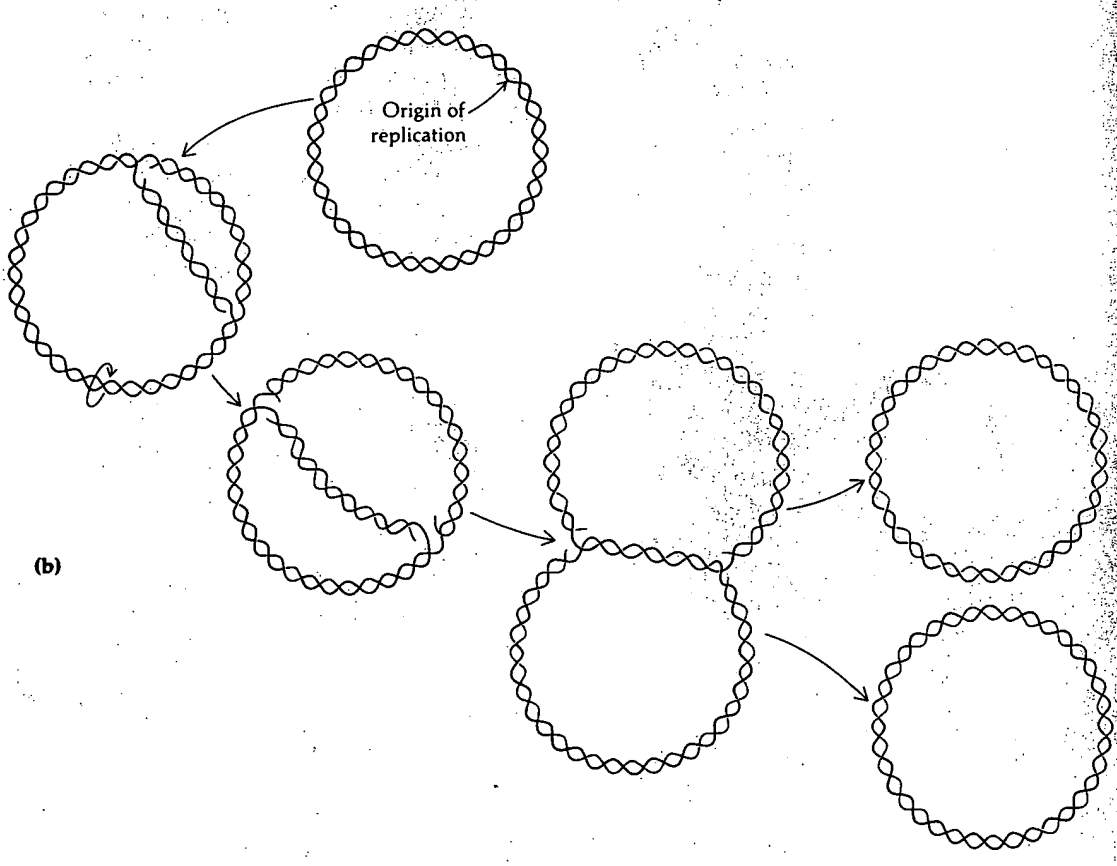


Figure 8–3 DNA replication. After the double helix separates, weak hydrogen bonds between nucleotides of the unspiraled strands break. Next, new complementary nucleotides are attached at the proper sites. Then hydrogen bonds are formed between complementary nucleotides and the process of replication is completed. Note that each daughter strand (gray) is a replica of one of the original (parent) strands (color).



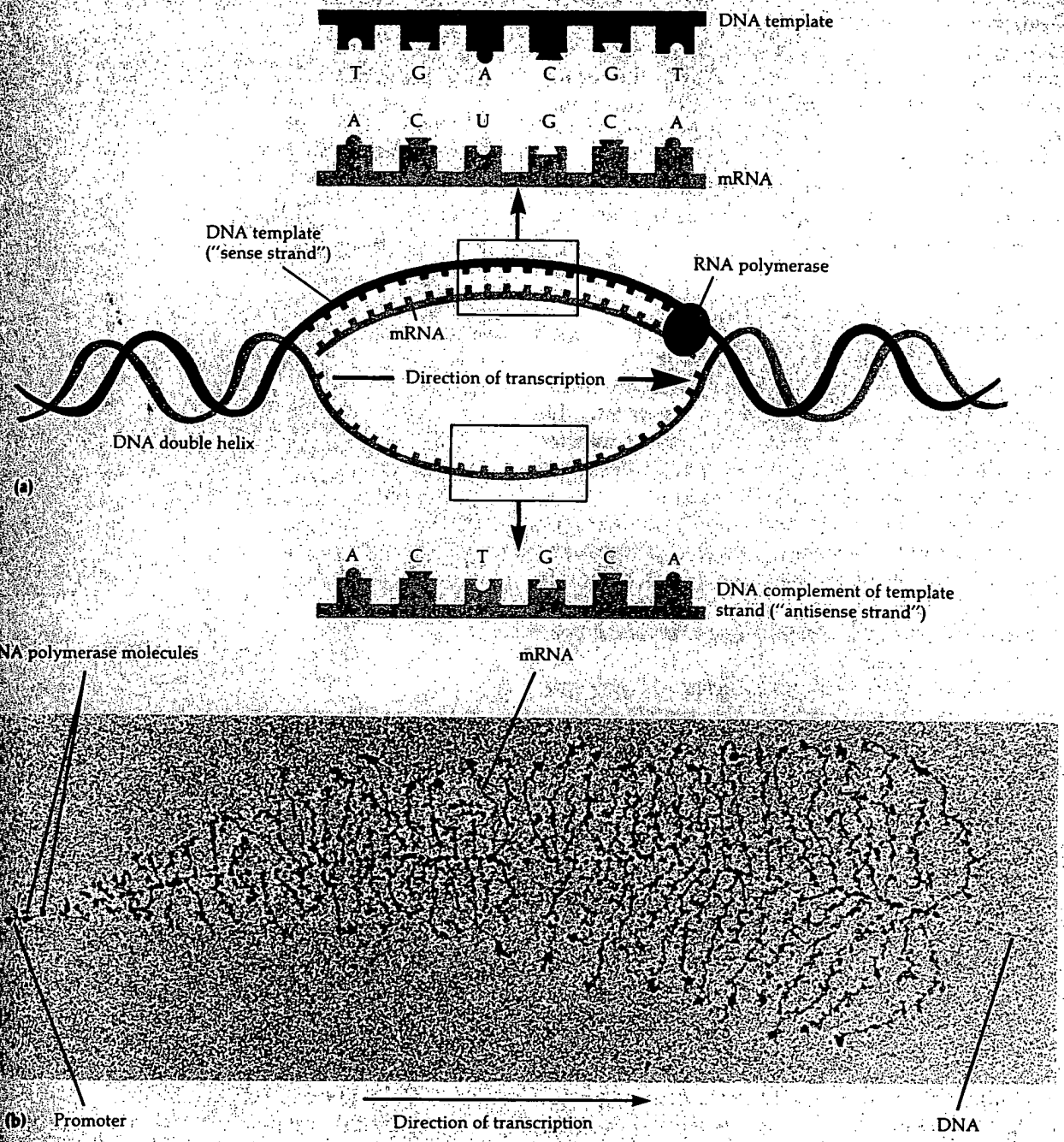
(a)





(b)

Figure 8–4 Replication of bacterial DNA. **(a)** Autoradiograph of the replication of an *E. coli* chromosome (corresponding diagram in the upper right). The arrows point to the two replication forks. The chromosome is about one-third replicated. Note that one of the new helices is crossed over the other one. **(b)** Diagrammatic representation of the bidirectional replication of a circular DNA molecule. The new strand is shown in color.



ure 8–5 Transcription. **(a)** Diagram of a partly uncoiled DNA double helix. nscription occurs in the unwound region of DNA. The upper box shows transcription, lower box the base sequence of the antisense DNA strand. **(b)** Electron micrograph ranscription from a single gene (×51,000). Many molecules of mRNA are being nesized simultaneously, starting at the promoter site. The longest mRNA molecules the oldest. Note the many RNA polymerase molecules working all along the DNA.

is the complement of the sense strand. The region where RNA polymerase binds tightly to DNA is known as the **promoter** site; this is where transcription begins. The region of DNA that acts as the end point for transcription is referred to as the **terminator** site; at this site the RNA polymerase and newly formed mRNA are released from the DNA. The DNA double helix then reforms.

In summary, then, the genetic information stored in DNA for protein synthesis is passed to mRNA during transcription.

Translation

The process of using the information in the nitrogenous base sequence of mRNA to dictate the amino acid sequence of a protein is called **translation.** The events involved in translation are described in this section and shown in Figure 8–6.

First, one end of the mRNA molecule becomes associated with a ribosome, the site of protein synthesis (Figure 8–6a). Ribosomes (see Chapter 4) consist of a special type of RNA, called **ribosomal RNA** or **rRNA,** and protein. Each ribosome consists of two subunits.

In solution in the cytoplasm are 20 different amino acids that may participate in protein synthesis. These 20 amino acids and their abbreviations are listed in Table 8–1. These amino acids are synthesized by the cell or taken up from the external medium. However, before the appropriate amino acids can be joined together to form a protein, they must be *activated.* Another type of RNA, called **transfer RNA** or **tRNA,** participates in this step (Figure 8–7a). For each different amino acid, there is a different type of tRNA. In the process of amino acid activation, a specific amino acid is attached to its specific type of tRNA. This attachment is accomplished by an enzyme plus energy from ATP (Figure 8–7b).

One part of the tRNA molecule has a sequence of three nitrogenous bases that matches up with its complementary triplet on an mRNA strand. Each set of three nitrogenous bases on mRNA is called a **codon.** Their complement, the three nucleotides in tRNA, is the **anticodon** (Figure 8–7c). During translation, the anticodon of a molecule of tRNA attaches to its complementary codon on mRNA. For example, a tRNA with anticodon UAC pairs with the mRNA codon AUG (Figure 8–6b). The tRNA molecule is also bringing along its amino acid. The pairing of codon and anticodon occurs only where mRNA is attached to a ribosome.

After the first tRNA, with its amino acid, attaches to mRNA, the ribosome moves along the mRNA strand and the second tRNA molecule, with its amino acid, moves into position (Figure 8–6c). The two amino acids are joined by a peptide bond, and the first tRNA molecule detaches itself from the mRNA strand (Figure 8–6c). It can now pick up another amino acid, for each tRNA can be used over and over again. As the proper amino acids are brought into line one by one, peptide bonds form between the amino acids and a polypeptide chain is formed (Figure 8–6d).

A special termination codon in the mRNA (called a **nonsense codon** because it does not specify any amino acid) signals the end of a polypeptide chain and its release from the ribosome (Figure 8–6e). The ribosome then comes apart into its two subunits.

As the ribosome moves along the mRNA, and before it completes translation of that gene, another ribosome may attach and begin translation of the

Table 8–1 The Twenty Amino Acids Found in Proteins and Their Abbreviations

Name	Three-Letter Abbreviation
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Cysteine	Cys
Glutamic acid	Glu
Glutamine	Gln
Glycine	Gly
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr
Valine	Val

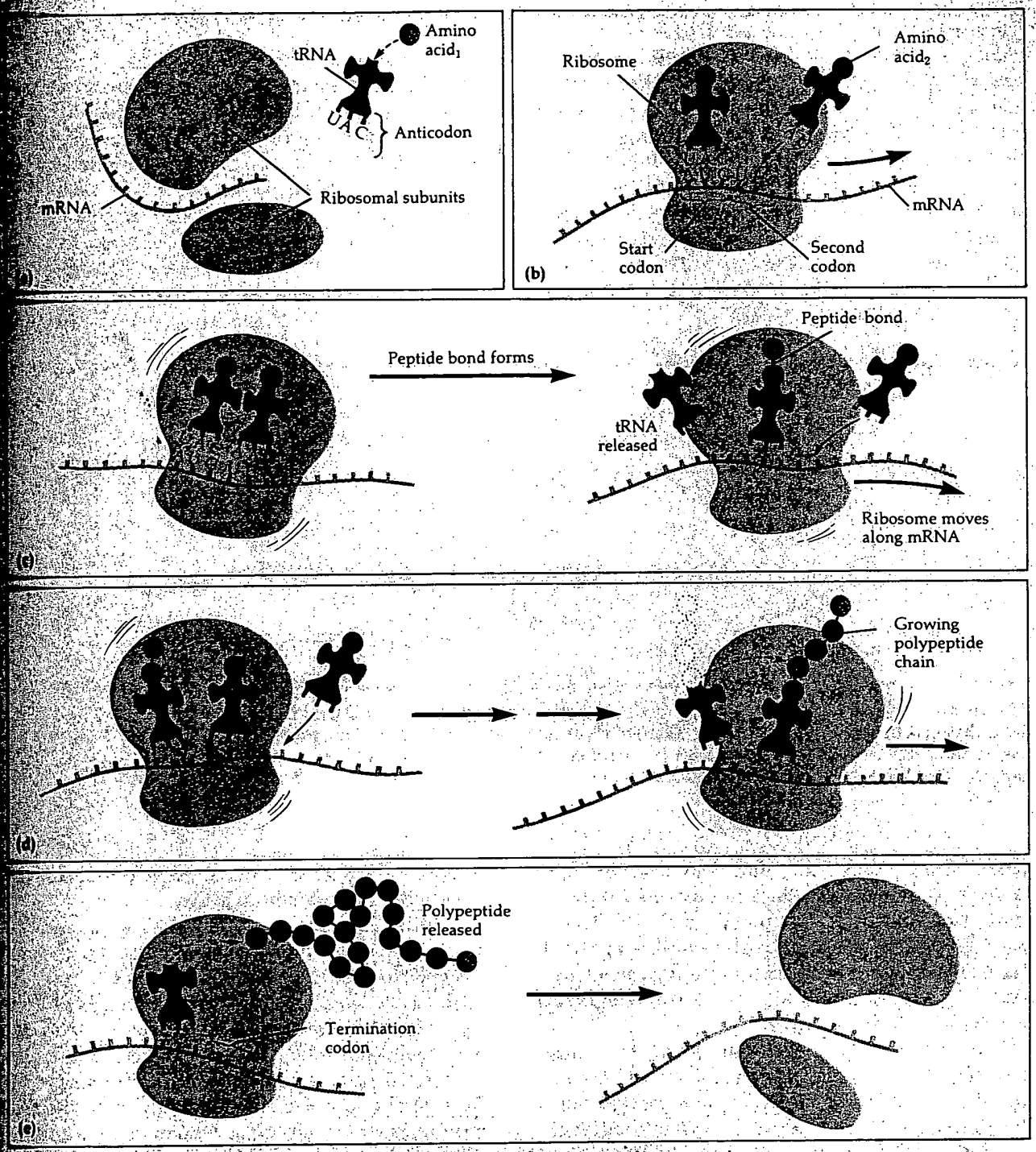


Figure 8–6 Translation. **(a)** Components needed for translation. **(b)** A tRNA carrying the first amino acid pairs with the start codon on the mRNA. **(c)** The second codon of the mRNA pairs with the tRNA carrying the second amino acid. The first amino acid is joined to the second by a peptide bond, and the first tRNA is released. **(d)** The ribosome moves along the mRNA to the next codon, and the process continues. (Nucleotide bases are shown only for the first two codons.) The chain of amino acids (polypeptide) grows. **(e)** When the ribosome reaches the termination codon, the polypeptide is released, the last tRNA is released, and the ribosome comes apart.

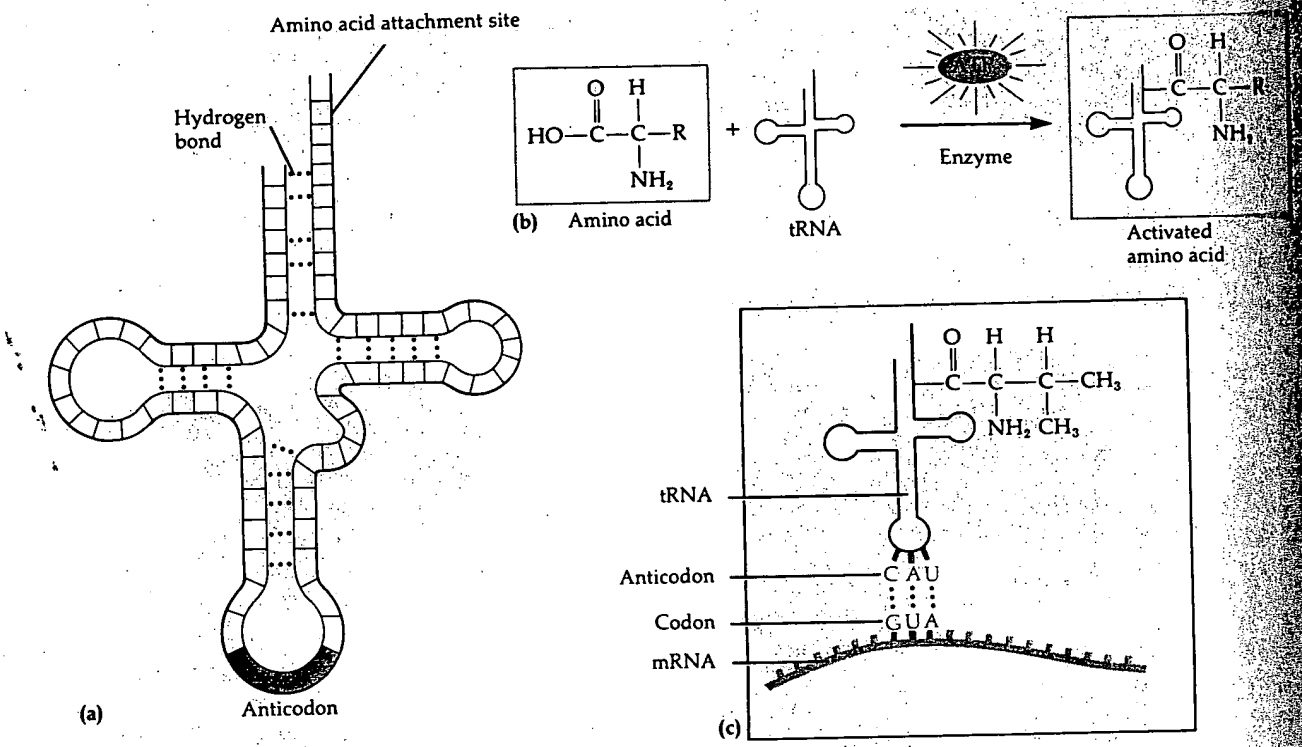


Figure 8–7 Transfer RNA. **(a)** Structure of tRNA. Each "box" represents a nucleotide. Note the regions of hydrogen bonding between base pairs, and the loops of unpaired bases. **(b)** Amino acid activation for attachment of an amino acid to tRNA. **(c)** The anticodon of tRNA pairs with its complementary codon on an mRNA strand.

same mRNA molecule, and then another, until there are a number of ribosomes attached at different positions to the same mRNA molecule. In this way, a single mRNA strand can be translated simultaneously into several identical protein molecules. An mRNA strand with several ribosomes attached is called a **polyribosome.**

In summary, the synthesis of a protein requires that the genetic information in DNA first be transferred to a molecule of mRNA by a process called transcription. Then, in a process called translation, the mRNA attaches to a ribosome, tRNA activates amino acids, and the amino acids are delivered to mRNA by tRNA according to complementary base pairings of codons and anticodons. The amino acids are then linked to form polypeptides. Proteins may be composed of one or more polypeptide chains, each of which may range in length from 50 to several hundred amino acids.

Since a typical polypeptide has about 300 amino acids, the typical mRNA must have about 300 codons and thus be about 900 bases long. As explained earlier, DNA is internally marked off in regions called genes, each region coding for a different kind of mRNA. A gene, therefore, can also be defined as a sequence of nitrogenous bases on DNA that codes for a functional product—and the product is almost always a protein, the exceptions being rRNA and tRNA. The DNA of *E. coli* is long enough to contain about 4000 genes and can therefore specify about 4000 different kinds of mRNA. In fact, *E. coli* has about 4000 different proteins. There are only a few copies of some proteins, but there are several thousand copies of other proteins

THE GENETIC CODE

The **genetic code** specifically refers to the relationship between the nitrogenous base sequence of DNA, the corresponding codons of mRNA, and the amino acids that the codons stand for (Figure 8–8). Note that there are 64 possible codons but only 20 amino acids. This means that most amino acids are signaled by several codons, a situation referred to as the **degeneracy** of the code. For example, leucine has 6 codons and alanine has 4 codons.

Of the 64 codons, 61 are sense codons and 3 are nonsense codons. **Sense codons** code for amino acids, while **nonsense codons** do not. Rather, they signal the end of synthesis for the protein molecule. These nonsense codons are UAA, UAG, and UGA, and they are the terminator codons referred to earlier. The initiator codon that starts the synthesis of the protein molecule is AUG, which is also the sense codon for methionine. The base sequence that specifies the promoter site on DNA is not translated.

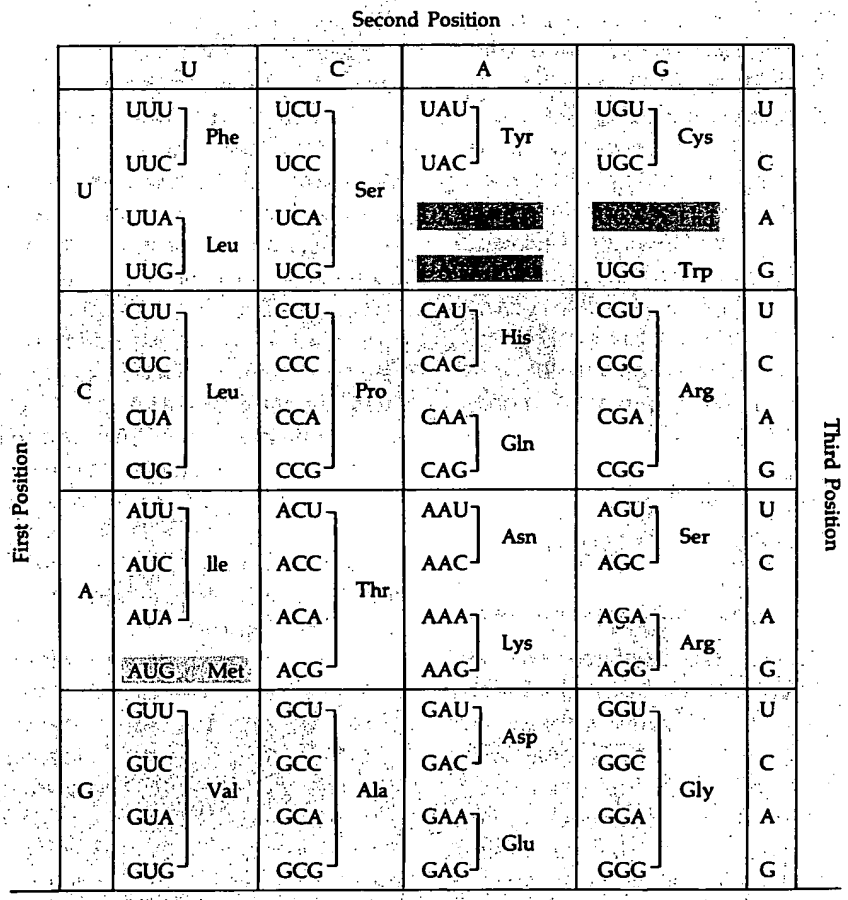


Figure 8–8 The genetic code. The three nucleotides in an mRNA codon are designated, respectively, as the first position, second position, and third position of the codon. Each set of three nucleotides specifies a particular amino acid, represented by a three-letter abbreviation (see Table 8–1). The codon AUG (which specifies the amino acid methionine) is the start signal for protein synthesis. The word *End* stands for the nonsense codons that serve as signals to terminate protein synthesis.

GENOTYPE AND PHENOTYPE

The **genotype** of an organism is its genetic makeup, the information that codes for all the particular characteristics of the organism. The genotype represents the *potential* properties, but not the properties themselves. **Phenotype** refers to the *actual, expressed* properties, such as the ability to perform a particular chemical reaction. The phenotype, then, is the external manifestation of the genotype.

In molecular terms, an organism's genotype is its collection of genes, its entire DNA, its blueprint for determining its unique characteristics. What, then, constitutes the organism's phenotype in molecular terms? To put it simply, an organism's phenotype is its collection of proteins. Most of a cell's properties derive from the structure and function of its proteins. In microorganisms, most proteins are either enzymatic, catalyzing particular reactions, or structural, participating in large functional complexes like membranes or ribosomes. Even those phenotypes that depend on structural macromolecules other than protein rely indirectly on proteins. For instance, the structure of complex lipid or polysaccharide molecules is derived from the catalytic activities of enzymes that synthesize, process, and degrade them. Although it is not completely accurate to say that phenotypes are due only to proteins, it is a useful simplification.

MUTATION: CHANGE IN THE GENETIC MATERIAL

A **mutation** is a change in the base sequence of DNA. We can reasonably expect that a change in the base sequence of a gene will cause a change in the gene product coded by that gene. When a gene mutates, the enzyme coded by the gene may become inactive or less active because its amino acid sequence has changed. Such a change in genotype may be disadvantageous or even lethal, because the cell loses a phenotypic trait it needs. Yet, a mutation may also be beneficial, for instance, if the altered enzyme coded by the mutant gene has a new enzymatic activity that benefits the cell.

Many simple mutations are neutral, the change in DNA base sequence causing no change in the activity of the product coded by the gene. A common example of a neutral mutation is the substitution of one nucleotide for another in the DNA. Because of the degeneracy of the genetic code, the resulting new codon may still code for the same amino acid. Even if the amino acid is changed, there may be no change in protein function if it is in a nonvital portion of the protein.

TYPES OF MUTATIONS

The most common type of mutation involving single base pairs is **base substitution,** where at one point in the DNA a single base is replaced with a different one. Then, when the DNA replicates, the result is a substituted base pair (Figure 8–9). For example, A–T may become substituted for G–C or C–G for G–C. If a base substitution occurs in a portion of the DNA molecule that codes for a protein, then the mRNA transcribed from the gene will carry an incorrect base at some position. When the mRNA is translated into protein, the incorrect base may cause the insertion of an incorrect amino acid in the protein. Thus, the base substitution in DNA can result in an amino acid substitution in the synthesized protein. This is known as **missense mutation** (Figure 8–10a and b).

Some base substitutions effectively prevent the synthesis of a functional protein by creating a terminator (nonsense) codon in mRNA before the protein is synthesized. Thus, only a fragment of the protein is synthesized. A base substitution resulting in a nonsense codon is called a **nonsense mutation** (Figure 8–10c).

Besides base pair mutations, there are also changes in DNA called **frameshift mutations.** Here, one or a few base pairs are deleted or added to DNA (Figure 8–10d). This can shift the "translational reading frame," that is, the three-by-three grouping of nucleotides recognized by the tRNA during translation. For example, inserting one base pair in the middle of a gene causes many amino acids downstream from the site of the original mutation to change. Frameshift mutations almost always result in an inactive protein product for the mutated gene. They lead to a long stretch of missense, and in most cases a nonsense codon will eventually be generated, terminating translation.

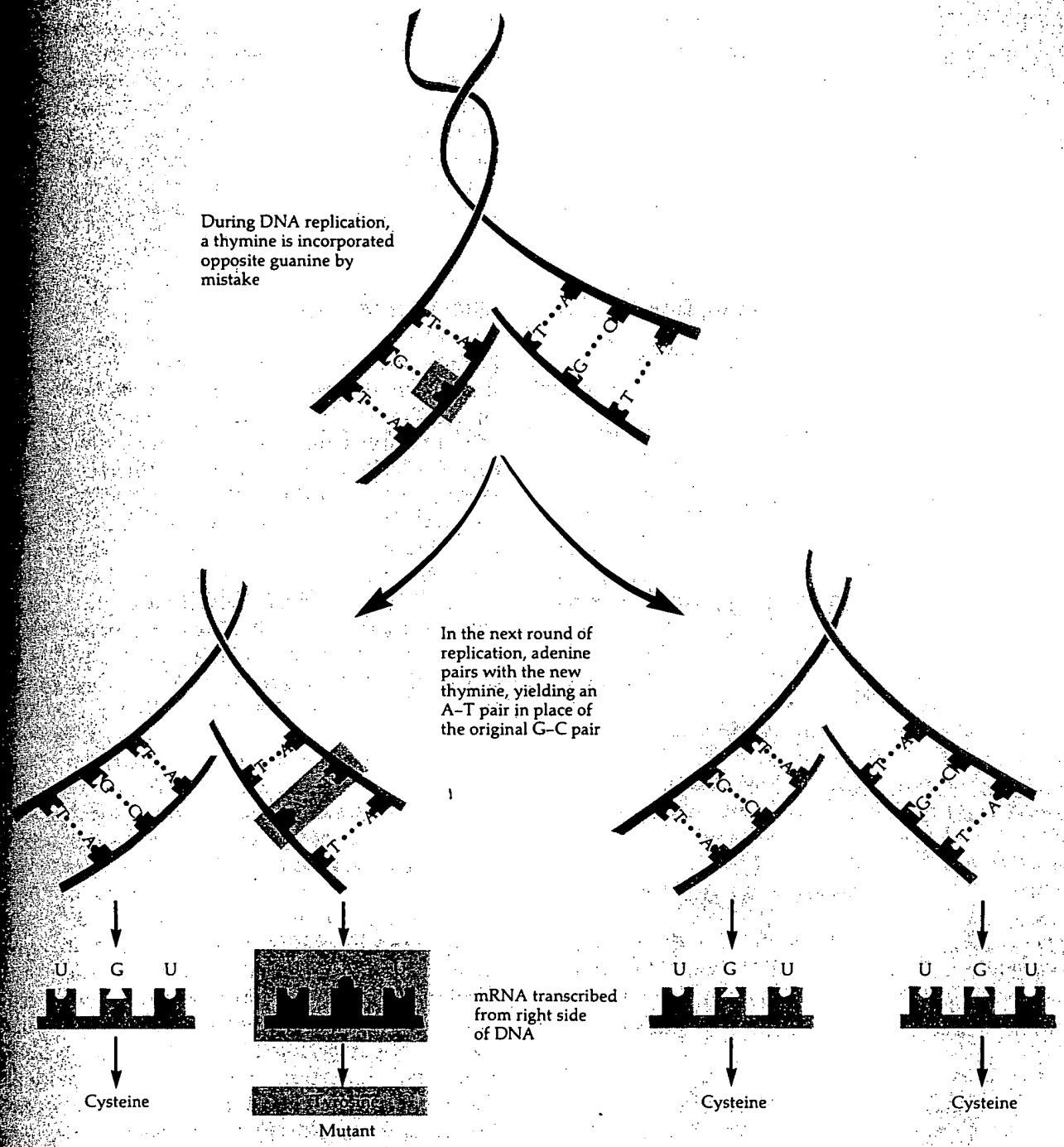


Figure 8–9 Base substitution, leading to base pair substitution.

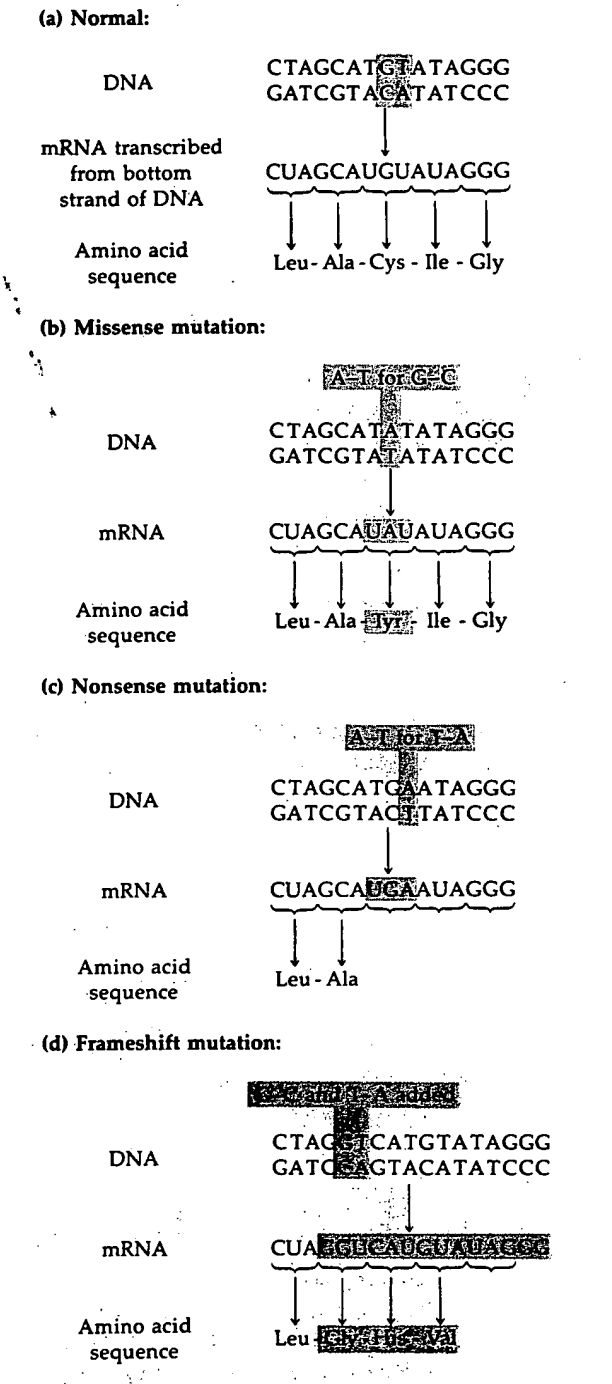


Figure 8–10 Types of mutations. **(a)** Normal DNA molecule. **(b)** Missense mutation. **(c)** Nonsense mutation. **(d)** Frameshift mutation.

MUTAGENESIS

Base substitutions and frameshift mutations may occur spontaneously because of occasional mistakes made during DNA replication. **Spontaneous mutations** are mutations that occur without known intervention of mutation-causing agents. Agents in the environment, such as certain chemicals and radiation, that directly or indirectly bring about additional mutations are called **mutagens.**

Chemical Mutagens

Among the many chemicals known to be mutagenic is *nitrous acid*. Figure 8–11 shows how exposure of DNA to nitrous acid can convert the base adenine (A) to a form that no longer base pairs with thymine (T) but with cytosine (C). When DNA containing such modified adenines replicates, one daughter DNA molecule will have a different base pair sequence than the parent DNA. Eventually some A–T base pairs of the parent will have been changed to G–C base pairs in daughter cells. Nitrous acid is thus an effective **base pair mutagen**, making a specific kind of mutational change in DNA. Like all mutagens, it does not select which gene it will mutate, but alters DNA at random locations.

Other chemical mutagens are **base analogs**, like *2-aminopurine* and *5-bromouracil*. These molecules are structurally similar to normal nitrogenous bases, but have slightly altered base-pairing properties. The 2-aminopurine (Figure 8–12a) is incorporated into DNA in place of adenine and can pair with thymine. The 5-bromouracil (Figure 8–12b) is incorporated into DNA in place of thymine and often pairs with guanine. When base analogs are given to growing cells, the analogs are metabolized and incorporated into cellular DNA in place of the normal bases at random places. Then, during DNA replication, they cause mistakes in base pairing. The wrongly paired bases may be faithfully copied during further DNA replication, and a base pair substitution results. Some antiviral drugs are base analogs.

Still other mutagens can cause small deletions or insertions instead of substitutions. For instance, *benzpyrene*, which is present in smoke and soot, is

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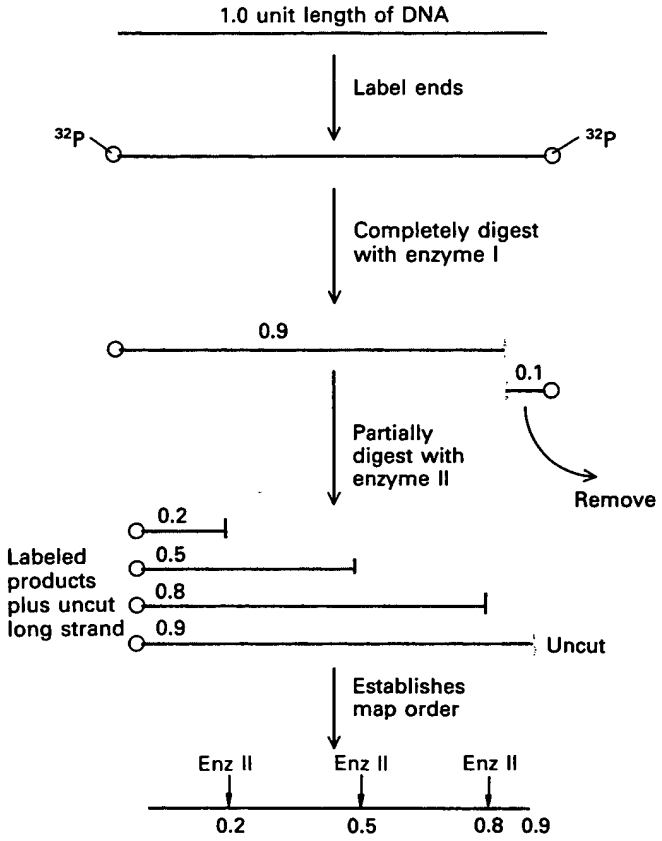
and 6-8). Fragments of 1–10 megabases (10^6–10^7 bp) are used to map the chromosomes of very large genomes, such as those of mouse and man.

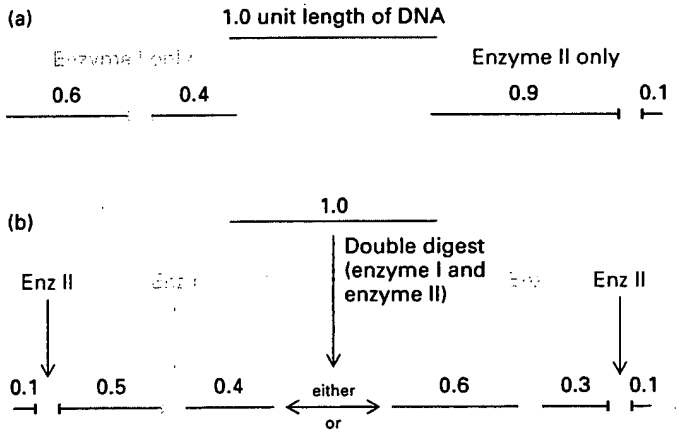
Digestion of DNA by restriction endonucleases, followed by simple electrophoretic separation of the fragments, has revolutionized chromosome mapping. The use of two or more restriction endonucleases on a pure DNA sample can show the order of the restriction sites in a DNA sample (Figure 6-22). Also, many sites can be located by partial digestion of terminally labeled DNA with only one enzyme (Figure 6-23). In these ways, it is possible to produce a map showing the order of the restriction sites in any region of DNA. An important application of restriction endonucleases is their use to cut off one end of a DNA sample that has been end-labeled so that DNA pieces labeled at the other end are available for further study (see Figure 6-23).

Southern DNA Blots The ability to divide DNA into reproducible pieces allows the restriction sites around a particular sequence of interest to be mapped. This possibility is realized in the laboratory by determining which restriction fragments hybridize to a specific labeled *probe* sequence technique called the *Southern blot* (after its originator, Edward Southern). DNA restriction fragments from a sample are separated by gel electrophoresis; their distribution in the gel is preserved as they are denatured and transferred by blotting to a solid substrate with a charged surface (usually a nitrocellulose filter). The filter is then exposed to a specific radioactive nucleic acid sequence (the probe). The blotted DNA fragments that are complementary to the probe hybridize with them, and their location on the filter can be revealed by autoradiography (Figure 6-24). This technique is so sensitive that a DNA sequence that appears only once in the human genome (about 1 part in 10^6) can be detected in as little as 5 μg of DNA (the DNA content of about 10^6 cells).

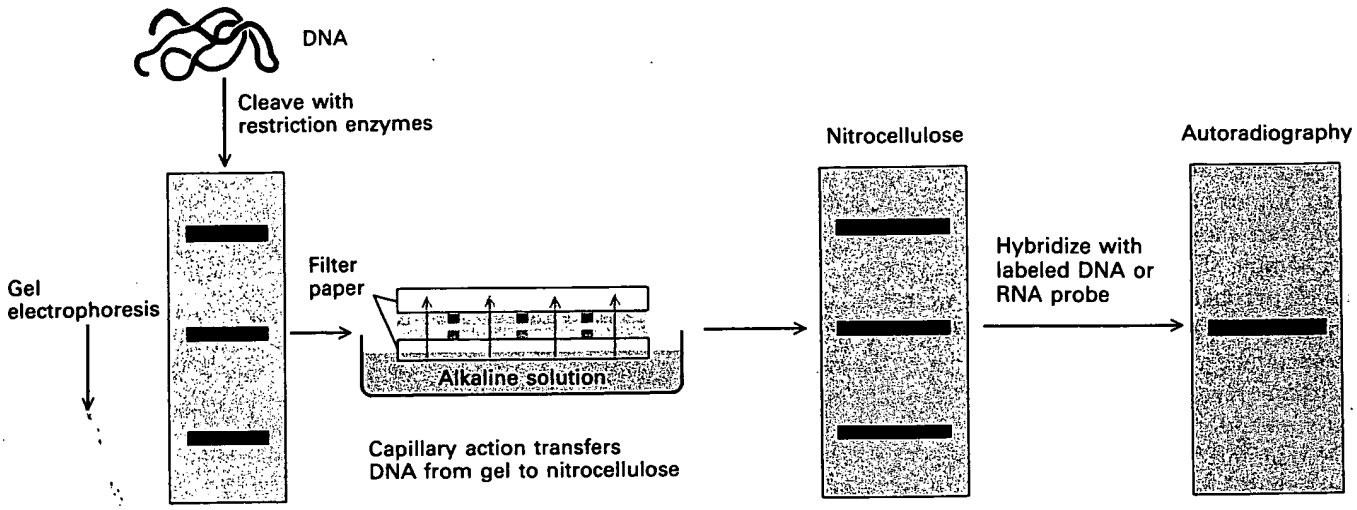
This test is widely used in genetic studies of humans, who do not, as a rule, breed within families. Consequently, the human population shows many genetic differences, or *genetic polymorphisms*. These variations are



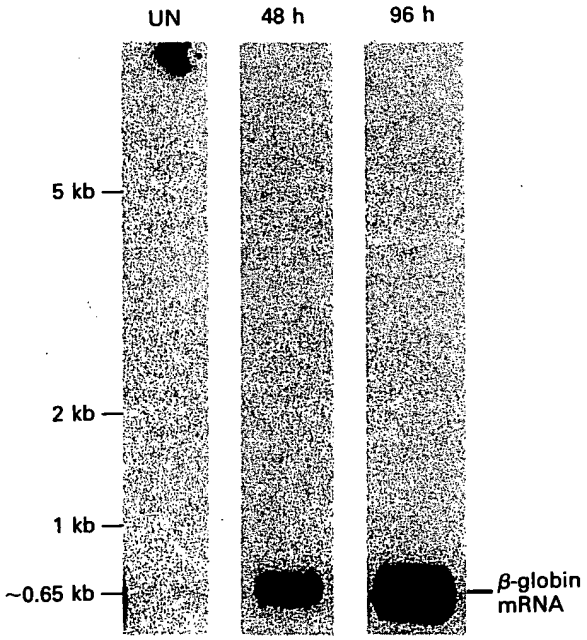
▲ **Figure 6-23** Mapping the multiple recognition sites of a restriction enzyme by partial digestion. DNA is labeled at its termini with ^{32}P, and fragments with *one* labeled terminus can be obtained by cutting off one end with an appropriate enzyme. The mapping procedure is applied to the remaining piece with a second enzyme. Complete digestion would produce only one labeled fragment (here, the 0.2-unit piece), but brief, partial digestion (in which the enzyme cuts each long piece only once, at most) produces a labeled fragment for each restriction site. From the lengths of the labeled pieces, the positions of enzyme II restriction sites can be inferred. [See H. O. Smith and M. Birnstiel, 1976, *Nuc. Acids Res.* 3:2387.]



▲ **Figure 6-22** Mapping the cleavage sites of two restriction enzymes with respect to one another. (a) When a given piece of DNA is exposed separately to two restriction enzymes (I and II), each cuts the DNA once. The lengths of the fragments are determined by gel electrophoresis. (b) Digestion with *both* enzymes is used to determine the relative positions of the cuts along the DNA. The fragment lengths identify the positions of the restriction sites for enzymes I and II with respect to the ends of the DNA and therefore with respect to each other. By continuing this process with different pairs of enzymes, the investigator can construct a detailed map of restriction sites.



▲ **Figure 6-24** The Southern blot technique for detecting the presence of specific DNA sequences. [See E. M. Southern, 1975, *J. Mol. Biol.* 98:508.]



▲ **Figure 6-25** The Northern blot technique for detecting the presence of specific mRNA molecules. Autoradiography shows the position of the complementary mRNA in the gel, and the density of the spot shows the amount of it. The photograph indicates the relative quantities in kilobases (kb) of β-globin mRNA in erythroleukemia cells at three different times: when cells are growing and have not started to make globin (lane UN, for "*un*induced"), and 48 and 96 h after they have been induced to stop growing and begin differentiating. The β-globin mRNA is barely detectable in growing cells but increases by a factor of more than 1000 in 96 h of differentiation. *Photograph courtesy of L. Kole.*

often indicated by the presence or absence of particular restriction sites in the DNA, called *restriction fragment-length polymorphisms.*

Northern (RNA) and Western (Protein) Blots The *Northern blot,* so-named because it is patterned after the Southern blot, is used to detect the presence of specific mRNA molecules. The RNA molecules in a sample are denatured by mixing them with an agent, such as formaldehyde, to prevent hydrogen bonds between base pairs (stems) and ensure that the RNA is in unfolded, linear form. The RNA sample (often the total RNA from cells) is then separated according to size by gel electrophoresis; as in a Southern blot, it is then transferred to a nitrocellulose filter to which the extended denatured RNA will adhere. The filter is exposed to a labeled DNA probe and subjected to autoradiography. The Northern blot indicates the amount as well as the presence and size of a specific mRNA in a sample and the procedure is widely used to compare the amounts of a specific mRNA in cells under different conditions (Figure 6-25).

Another bit of laboratory jargon that has become a widely accepted name for a common technique is the *Western blot.* In this procedure, a one- or two-dimensional electrophoretic separation of proteins is carried out and the protein is then transferred, or blotted, to nitrocellulose. The nitrocellulose sheet can be exposed to radioactive antibodies against a particular protein; autoradiography reveals the presence of that protein.

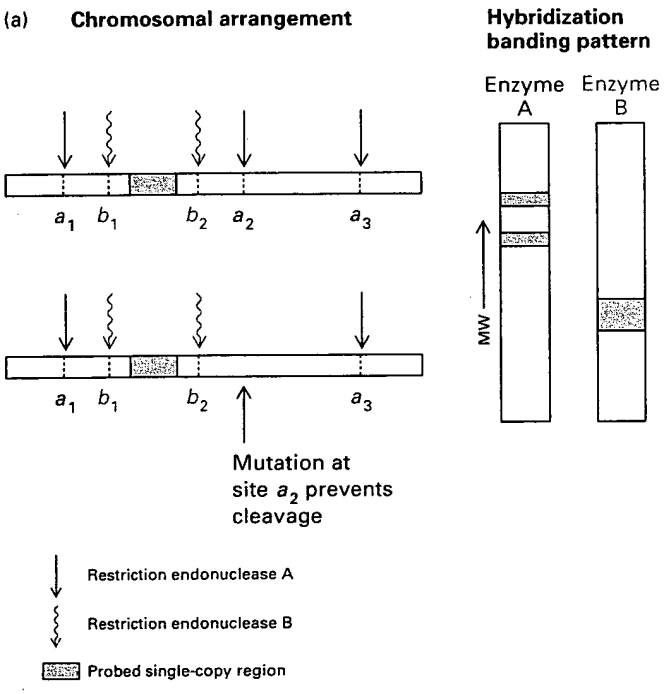
Band Analysis of S1 Digests An important method for measuring the length of complementary sequences in two nucleic acids employs the endonuclease S1, an en-

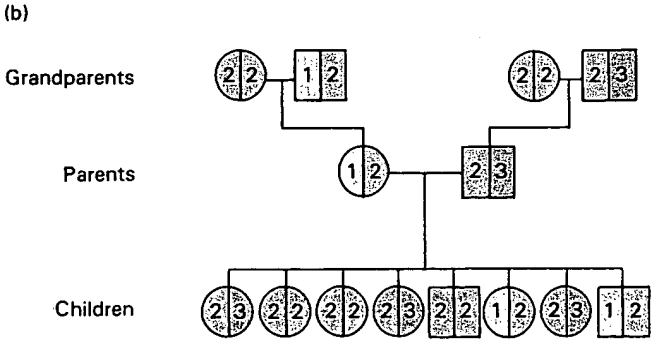
mouse which contains parts of chromosomes 1 and 2 in humans. Many other such *syntenic* stretches exist between mice and humans, indicating that our chromosomes are a patchwork of recombined segments from our mammalian forbears. Although fewer genes have been located in other primate chromosomes, from what is known, human and chimpanzee chromosomes are quite similar in most gene locations. Thus the eukaryotic chromosome behaves genetically, and appears visually, as a linear structure containing a single DNA duplex.

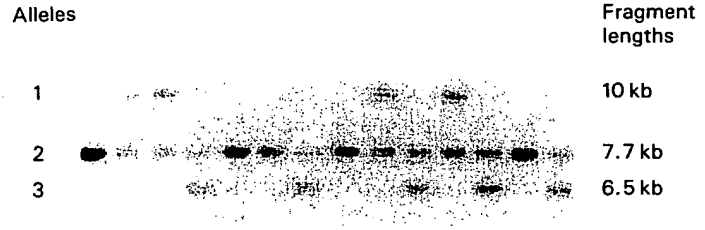
Human Chromosomes Can Be Mapped Based on Restriction Fragment Length Polymorphisms (RFLPs)

The existence of distinctive chromosomal banding patterns provides a basis for physical maps of chromosomes. In the case of human chromosomes, however, mapping based on banding patterns is very rough because each Q or R band contains a relatively large proportion (5–10 percent) of a chromosome. And in situ hybridization, with labeled nucleic acid probes prepared from cloned DNA, can locate genes only in a general region of a chromosome. In recent years, developments in molecular genetics have provided new methods of physically mapping genetically inherited sites. The use of these techniques has already resulted in maps with markers spaced approximately 0.5 to 2.5 $\times$ 10^7 base pairs apart.

The first of these genetic maps of human chromosomes was constructed by taking almost 2000 separate cloned DNA segments and determining by Southern blot analysis whether differences between two DNA samples from a group of families were similar or not. In a Southern blot analysis, a *restriction endonuclease* that recognizes a specific base sequence of 4–8 nucleotides in length is used to digest a DNA sample; the resulting fragments are then separated by electrophoresis and reacted with a labeled cloned DNA sample (the probe) to determine the size of the hybridizing fragments. If several restriction enzymes are used to cut the DNA, differences among samples from different individuals usually can be found because the enzymes are sequence-dependent, and a change in sequence at the restriction site results in different sized fragments (Figure 9-7a). These are called *restriction fragment*







◀ **Figure 9-7** Analysis of restriction fragment length polymorphisms (RFLPs). (a) In the example shown, DNA is treated with two different restriction enzymes, which cut DNA at different sequences. The resulting fragments are subjected to Southern blot analysis; that is, they are separated by electrophoresis, transferred to nitrocellulose, and detected by hybridization with a radioactive probe, which binds to the indicated DNA region (green). Since no differences between the two chromosomes occur in the sequences recognized by the B enzyme, only one fragment is produced, as indicated by a single hybridization band. However, treatment with enzyme A produces fragments of two different lengths (two bands are seen), indicating that a mutation has caused the loss of one of the *a* sites. (b) RFLP analysis of the DNA from eight children, their parents, and grandparents detected the presence of three alleles for a region known to be present on chromosome 5. The DNA samples were cut with the restriction enzyme *Taq*I and analyzed by the Southern blot procedure. In this family, this region exists in three allelic forms characterized by *Taq*I sites spaced 10, 7.7, or 6.5 kb apart. Each individual has two alleles; some contain allele 2 (7.7 kb) on both chromosomes, and others are heterozygous at this site. *After H. Donis-Keller et al., 1987,* Cell *51:319.*

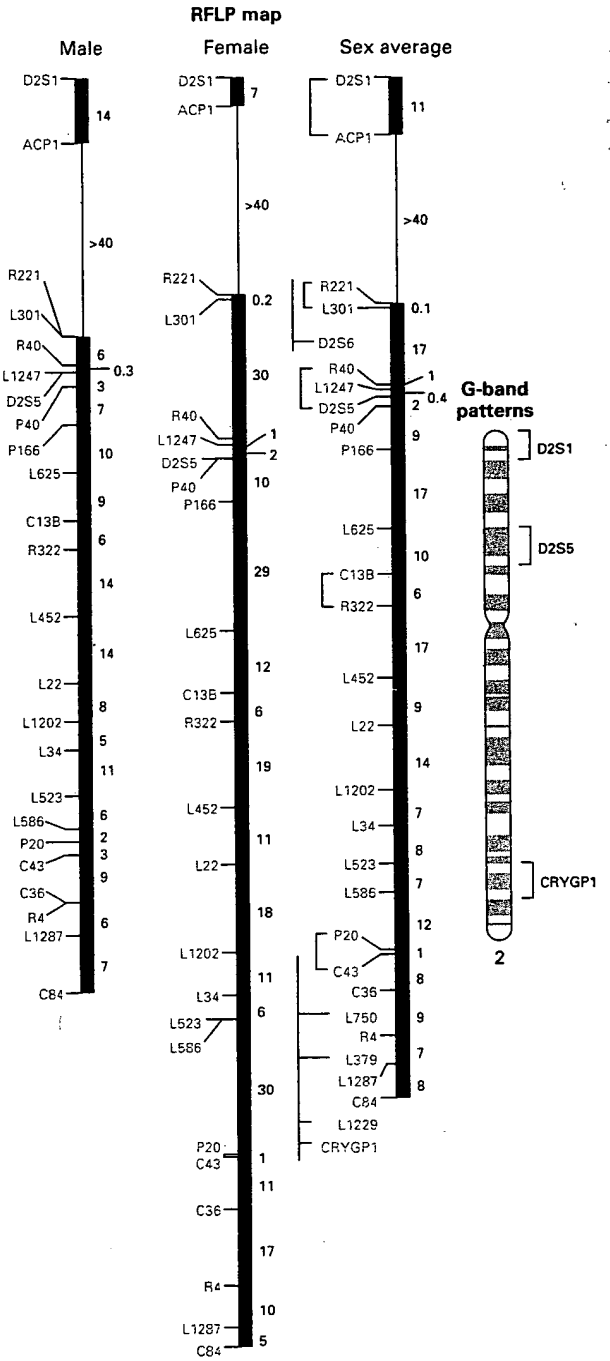
length polymorphisms (RFLPs). When such a polymorphism is found, it can be traced within a family and shown to be inherited in a Mendelian fashion (Figure 9-7b).

By now well over a thousand RFLPs have been identified in human DNA (and more are reported each month), providing a large number of physically identified genetic markers. Inheritance of the polymorphisms for these markers in families where DNA is available for three generations allows linkage maps between different RFLPs to be established. Computer comparisons of the linkage data are often used to determine which chromosome contains the DNA exhibiting a particular RFLP (Figure 9-8). Because many already identified genes in humans have been mapped to chromosomes or parts of chromosomes, further analysis of the segregation of RFLPs with known human traits can often position the RFLP relative to a known gene on a particular chromosome. For example, the gene responsible for *cystic fibrosis,* a disease that results in chronic infections because of poor respiratory tract draining, was known from linkage studies to reside on human chromosome 7. The locations of RFLPs on either side of the gene, coupled with molecular cloning techniques, allowed this gene to be cloned even though the biochemical nature of the protein was unknown. Chromosomal positions can also be determined by using labeled cloned DNA probes bearing RFLP sites as a probe in in situ chromosomal hybridization.

Autonomously Replicating Sequences, Centromeres, and Telomeres Are Required for Replication and Stable Inheritance of Chromosomes

So far we have emphasized that eukaryotic chromosomes are linear structures composed of single DNA molecules. Although chromosomes differ in length and number among species, they all behave similarly at the time of cell division. At mitosis the attached sister chromatids become aligned on the metaphase plate; they then separate at the centromeres, and one chromatid of each metaphase chromosome is distributed to each daughter cell. Recent recombinant DNA research with yeast cells has identified all of the chromosomal elements that are necessary for equal segregation of sister chromatids to occur. The culmination of this work has been construction of artificial yeast chromosomes. In order to duplicate and segregate correctly, chromosomes must contain three functional elements: (1) special sequences involved in the initiation of DNA replication; (2) the centromere; and (3) the two ends, or telomeres.

Autonomously Replicating Sequences If yeast cells lack a particular gene (e.g., one of the genes that encode an enzyme for synthesis of the amino acid leucine), they can be transfected with cloned plasmids containing the



▲ **Figure 9-8** Genetic map of human chromosome 2 based on Southern blot analysis of restriction fragment length polymorphisms (RFLPs) and crossover frequencies during meiosis. The location of the RFLPs is shown by blue numbers on the left. The red numbers on the right show crossover frequencies (%) by adjacent markers. Thin lines near the tops of the maps represent markers that are sufficiently distant that they appear to be unlinked, but are known to be on chromosome 2. As in other species, crossing over occurs at different locations in males and females. Comparison of this map with the G-band pattern of chromosome 2 illustrates the greater sensitivity of RFLP analysis for mapping genetic loci. A map similar to this is available for every human chromosome. *After H. Donis-Keller et al., 1987,* Cell *51:328.*

Unfortunately, lung cancer is the only major human cancer for which a clear-cut risk factor has been identified. Animal fat is thought to be a risk factor for colon and breast cancer, and many viruses and chemicals have minor cancers; however, hard evi- us avoid breast cancer, colon can- leukemias, and others is generally

Rare ities to Cancer Point to Antioncogenes

A corollary to the belief that multiple interacting events in our environment are the major risk factors for cancer is the belief that genetic inheritance plays only a small role in carcinogenesis. This proposition is supported by the finding that people who migrate to a new environment take on the profile of cancers in their new environment within a generation. For instance, when Japanese citizens move to California, they rapidly lose the oriental propensity toward stomach cancer and soon show the occidental propensity toward breast cancer.

Genetic inheritance does, however, play some role in human cancer. Certain inherited genes increase the probability that an individual will get a specific tumor to almost 100 percent. A classic case is retinoblastoma, which is, like most inherited tumors, a disease of childhood. Children who inherit a single defective copy of the *RB* gene, often seen as a small deletion on chromosome 13, will come down with an average of three retinoblastoma tumors, each derived from a single transformed cell. Because the developing retina contains about 4×10^6 cells, only about 1 in 10^6 cells actually becomes a tumor cell. This finding suggests that even with its highly dominant inheritance, the *RB* gene is acting recessively at the cell level, and that a second event is needed to bring on the transformed state. The second event is now known to be the deletion or mutation of the normal *RB* gene on the other chromosome. Rare, somatic events can cause this deletion either by loss of all or a segment of the chromosome or by small alterations in the remaining *RB* gene. When chromosomal loss occurs, it is balanced by duplication of the affected chromosome.

Although the exact function of the *RB* gene product is not known, it is a nuclear protein and thus may affect transcription. Because its loss is the cause of malignant transformation, it is thought to act negatively, to suppress the oncogenic potential of other proteins. It is thus considered an *antioncogene*. Its targets are not known but could be transcriptional activators. An intriguing finding is that the RB protein binds tightly to DNA viral oncogene products. Perhaps these oncogene proteins cause transformation by tying up the RB protein, thus releasing the oncogenic potential of those proteins that RB ordinarily regulates.

Cancer induction by deletion of a genetic region, rather than by activation of an oncogene, appears to occur in a variety of childhood tumors associated with inherited defects. These are all rare tumors, but it is believed that deletional carcinogenesis plays a wider role than is now evident. There may well be many antioncogenes, and their deletion may be an important determinant of the progression of human cancers.

Studies of human tumors have indicated that inheritance is only rarely a crucial risk factor; however, whether inheritance might be a minor risk factor in many cancers is still being debated. Genetic propensity might, for example, explain why some smokers get lung cancer and others do not.

Summary

Cancer represents a fundamental aberration in cellular behavior that involves many aspects of molecular biology. To become a cancer cell, a normal cell must undergo many significant changes. It must continue to multiply when normal cells would be quiescent; it must invade surrounding tissues, often breaking through the basal laminas that define the boundaries of tissues; and it must spread through the body and set up secondary areas of growth in a process called metastasis. All of the various cell types of the body can give rise to cancer cells. Cancer cells are usually closer in their properties to immature normal cells than to more mature cell types. The retention of malignant properties by cancer cells grown in culture shows that the alteration from a normal cell to a cancer cell is caused by events within the cancer cell itself.

By growing cells in culture, experimenters can freely manipulate them to learn about the properties that distinguish normal cells from cancer cells. Carcinogens, cancer-causing agents, can also be used to alter cultured cells to produce full-fledged cancer cells in a process called transformation. Transformed cells differ from normal cells in many ways, including cell-growth control, cell morphology, cell-to-cell interactions, membrane properties, cytoskeletal structure, protein secretion, gene expression, and mortality (transformed cells can grow indefinitely). These alterations in cell behavior are not wholly independent, but their interrelationships remain obscure.

The transformation of a cell from normal to malignant can be the consequence of the expression of one or a few genes, called oncogenes. Oncogenes are formed in cells or carried into cells by transforming agents, of which we recognize three main types: viruses, chemical carcinogens, and radiation. Certain DNA viruses and RNA-containing retroviruses can transform cells by permanently integrating new genes into the DNA of infected cells. The DNA-containing papovaviruses carry oncogenes that can cause cell transformation by inducing in

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